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A Rapid and Reproducible Protocol for the Purification of Insect Storage Protein

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ABSTRACT: Insect storage proteins are synthesised by the fat body and released into the haemolymph during larval stage and then they are sequestered by the fat body at prepupal and early-pupal stage and stored as protein granules. We report here a protocol for the purification of storage protein from the larval haemolymph of rice moth, *Corcyra cephalonica*. This involves use of a Sephadex G 50–150, a gel filtration column followed by an ion exchange DEAE Sephacel column. The protocol is simple, rapid and requires small quantity of starting material (haemolymph proteins) and gives good yield of storage protein with high fold of purification. The protocol has also been successfully employed for the purification of storage protein from the haemolymph of other lepidopteran insect silk worm, *Bombyx mori*.

KEYWORDS: purification, storage protein, fat body, insect.

INTRODUCTION

Storage proteins (SPs) are synthesized by the fat body predominantly during the larval development and are released into the haemolymph. During the last larval instar of holometabolous insects, these proteins nearly account for 70–80% of the total soluble protein by weight (Kanost *et al.*, 1990; Telfer and Kunkel, 1991). Shortly before pupation, they are sequestered by the fat body where they accumulate as dense protein granules. The SPs serve as a reserve pool of amino acids required for the remodeling of tissues and for deposition of cuticle during pupal-adult transformation (Levenbook, 1985; Bean and Silhacek, 1989; Chrysanthis *et al.*, 1994; Haunerland *et al.*, 1996; Burmester and Scheller, 1999).

Our laboratory has identified SPs in *C. cephalonica* and *Chilo partellus* and focused on the sequestration of these proteins by the fat body and male accessory reproductive glands (MARG) (Ismail and Dutta-Gupta, 1990, 1991; Kirankumar *et al.*, 1997).

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Sequestration of SP is stimulated by 20-hydroxyecdysone both in fat body as well as in MARG (Ismail and Dutta-Gupta, 1990, 1991; Ismail *et al.*, 1993). The uptake of SP is a receptor mediated process and the receptor has been recently identified in the fat body cells of *C. cephalonica* (Kirankumar *et al.*, 1997).

Storage proteins also known as hexamerins are high molecular weight multimers, usually hexamers. Each monomer is composed of subunits in the mass range of 70–90 kDa. This characteristic feature seems to be well retained in the several orders of holometabolous insects as well as in some orders of hemimetabolous insects (Telfer and Kunkel, 1991; Levenbook, 1985; Haunerland *et al.*, 1996; Rahbe *et al.*, 1990; Rehn and Rolim, 1990; Martinez and Wheeler, 1993; Tojo and Yoshiga, 1994).

In *C. cephalonica*, native SP is a hexamer (500 kDa) consisting of three subunits with mass of 86 kDa (SP1), 84 kDa (SP2) and 82 kDa (SP3). Based on the abundance of SPs in haemolymph during the last instar larval stage, its high molecular weight and its ability to bind to anion exchange matrix (Kirankumar *et al.*, 1997), we report here a rapid protocol for the purification of storage protein from the larval haemolymph of *C. cephalonica*.

MATERIALS AND METHODS

Insects

Corcyra cephalonica (Stainton).

It is commonly known as rice moth. The insects were reared in the culture room at 26 ± 1 °C temperature and 70 ± 5% relative humidity under a 14 : 10 h light-dark period. For the present study, mostly the late-last instar larvae (LLI) were used. The larval stages were identified by head capsule size and body weight as stated in our earlier publications (Ashok and Dutta-Gupta, 1988; Lakshmi and Dutta-Gupta, 1990). For immunological experiments haemolymph was collected from early, mid and late-last instar larvae.

Bombyx mori.

Third instar larvae of *Bombyx mori* (pure Mysore strain) were obtained from local breeding centre and reared in an insect culture room at 26±1°C, 70 ± 5% relative humidity and 14 : 10 h light-dark period on fresh mulberry leaves. Staging of insect was done based on their age after the fourth ecdysis and 9–10 days old were used as late-last instar for the present study.

Chemicals

Most of the chemicals used in the present study were procured from Sigma Chemicals Co., St. Louis MO., USA. DEAE Sephacel was obtained from Pharmacia Biotech. and nitrocellulose membrane from Schleicher & Schuell, Germany. Enzyme conjugated secondary antibody was procured from Bangalore Genei, India.

Collection of haemolymph

The larvae were narcotized on ice. The prolegs of the narcotized larvae were cut with a fine sharp scissors and the oozing haemolymph was collected with the help of capillary tube into 1.5 ml microfuge tubes which were prerinsed with 0.01% phenylthiourea in order to prevent tyrosinase activity and melanisation. All the haemolymph samples thus obtained were diluted with 10 mM Tris-HCl buffer (pH 7.4), and were centrifuged at 4000× g for 2 min at 4 °C to sediment the haemocytes. The supernatant was collected and stored at −20 °C till further use.

Protein estimation

Protein estimation was carried out according to Bradford's microprotein assay method (Bradford, 1976). The protein concentration of the sample was determined from standard curve drawn using BSA (fraction V).

Preparation of Sephadex G 50–150 gel filtration column

Sephadex G 50–150 was swelled in excess of double distilled water at room temperature. The swollen matrix was washed, equilibrated with 10 mM Tris-HCl (pH 7.4) and packed in a column to a bed volume of 110 ml after degassing. The void volume was calculated using blue dextran.

Preparation of DEAE Sephacel ion exchanger column

DEAE Sephacel a beaded ion exchanger is supplied as a preswollen suspension with 20% ethanol as preservative. The matrix was washed with excess of double distilled water to remove the ethanol, then it was equilibrated using 10 mM Tris-HCl (pH 7.4) and packed in a column to the bed volume of 30 ml after degassing.

Gradiant elution

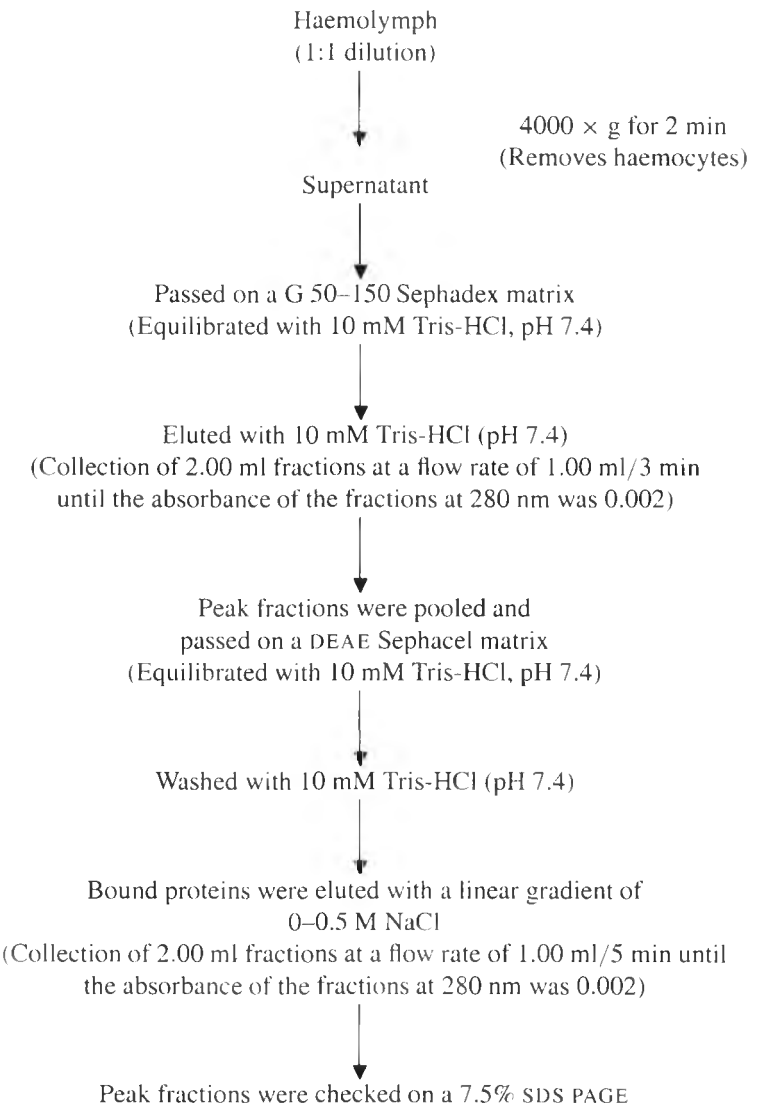
A simple two chambered glass apparatus of 100 ml capacity was used for forming linear concentration gradient. A linear gradient of 0–0.5 M NaCl was made by taking double distilled water in one chamber and 0.5 M NaCl in the other. The outlet from the water-containing chamber was connected to the DEAE Sephacel column by a rubber tube.

Protein electrophoresis

SDS-PAGE.

Tris-glycine SDS-PAGE was carried out according to the procedure of Laemmli (1970). The proteins were separated on 2.1% stacking gel and 7.5% resolving gel. The electrophoresis was carried out at constant voltage. Four to five μg of protein was loaded in each lane for separation.

The purification protocol devised in the present study has been summarized in the following flow chart



The SPs from *C. cephalonica* as well as *B. mori* were purified four times using above mentioned protocol and the results obtained were reproducible.

Silver staining.

The proteins in polyacrylamide gels were stained using the procedure of Blum *et al.* (1987) with minor modifications.

TABLE 1A. Purification of storage protein from *C. cephalonica* haemolymph. The results presented are mean of four independent purifications

Steps	Protein (mg)	Yield (%)
Haemocyte free crude haemolymph	6	100.0
Sephadex G 50–150 elution	3.8 ± 0.2	63.4–66.7
DEAE Sephacel elution	1.4 ± 0.1	23.4–25.0

Molecular weight determination.

Molecular weights of electrophoretically separated polypeptides were determined by coelectrophoresing high molecular weight marker proteins.

Western blotting and immune staining

Protein blotting was carried out according to the protocols of Towbin *et al.* (1979). The blot was probed using the primary antibody raised against purified SPs of *C. cephalonica* in rabbit. The detection was made using ALP conjugated secondary antibody.

RESULTS

Purification of SPs from *C. cephalonica* haemolymph

(a) Elution profile of haemolymph proteins on Sephadex G 50–150 matrix and SDS-PAGE of peak fractions eluted from this matrix

The haemocyte free diluted haemolymph from *C. cephalonica* (protein concentration of 1 mg/50 μl) was passed on Sephadex G 50-150 column equilibrated with 10 mM Tris-HCl (pH 7.4) at room temperature. The protein was eluted with 10 mM Tris-HCl buffer (pH 7.4) at a flow rate of 1 ml/2.5 min. Two ml fractions were collected until the absorbance of the eluates at 280 nm reached 0.002 (Fig 1a). Each fraction that constituted the peak was checked on electrophoregram (Fig. 1b). At this step of purification, almost 80% of the contaminant proteins were eliminated and the peak fractions contained higher amounts of SPs. When 6 mg of haemocyte free crude haemolymph protein was loaded on to this matrix, the peak fractions recovered accounted for 3.8–4.0 mg protein (Table 1a). These results also revealed that the SPs get eluted in the void volume.

(b) Elution profile of SPs on DEAE Sephacel matrix

With an objective to purify the SPs further the peak fractions obtained from Sephadex G 50–150 matrix were pooled (approximately 3.8–4.0 mg protein) and loaded on to the DEAE Sephacel column. The column was washed with 10 mM Tris-HCl buffer (pH 7.4) and the flow through fractions were collected until the absorbance at 280 nm was found to be 0.002. It was interesting to note that the flow through

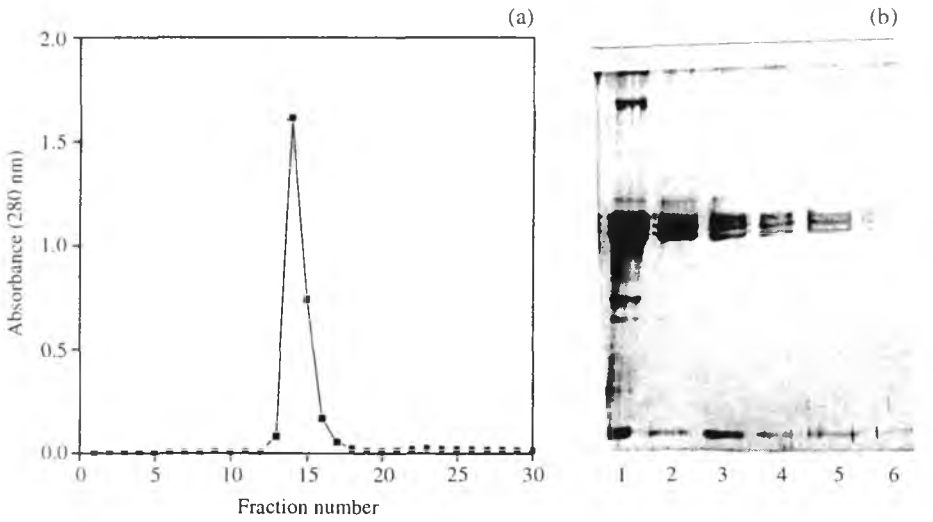


FIGURE 1. (a) Elution profile of haemolymph proteins of *C. cephalonica* on Sephadex G 50–150 column; (b) SDS-PAGE of peak fractions of *C. cephalonica* haemolymph proteins eluted from Sephadex G 50–150 column. Lane 1: crude haemolymph, lanes 2–6: 13–17th fractions.

TABLE 1B. Purification of storage protein from *B. mori* haemolymph. The results presented are mean of four independent purifications

Steps	Protein (mg)	Yield (%)
Haemocyte free crude haemolymph	6	100.0
Sephadex G 50–150 elution	3.1 ± 0.2	51.7–55.0
DEAE Sephacel elution		
Peak I	1.6 ± 0.2	26.7–30.0
Peak II	0.6 ± 0.1	10.0–11.7

fractions had negligible absorbance, suggesting that the proteins loaded were bound to the DEAE Sephacel matrix. Hence, it became necessary to elute them with higher concentration of salt. Since the matrix was an anionic exchanger, NaCl was the choice buffer. A linear gradient of 0–0.5 M NaCl was found to be the optimal concentration that eluted SPs into pure fractions (Fig. 2a). The peak fractions were pooled and analyzed on an electrophoregram (Fig. 2b). The results revealed that peak fractions contained SPs in purified form. This gel (Fig. 2b) was over stained, with a view to visualize minor contamination, and the results revealed the presence of only SPs with high fold of purification. Figure 3 shows the comparative profile of *C. cephalonica* haemolymph proteins at various steps of purification. Table 1a represents the recovery of protein at each step of purification from *C. cephalonica* haemolymph.

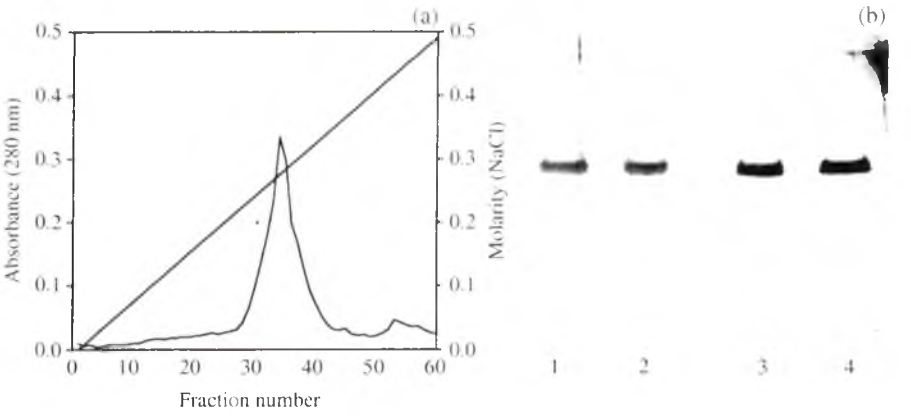


FIGURE 2. (a) Elution profile of SPs of *C. cephalonica* from DEAE Sephacel column using a linear gradient of 0–0.5 M NaCl; (b) SDS-PAGE of peak fractions of *C. cephalonica* proteins eluted from DEAE Sephacel column using a linear gradient of 0–0.5 M NaCl. Lanes 1–4: 33–36th fractions.

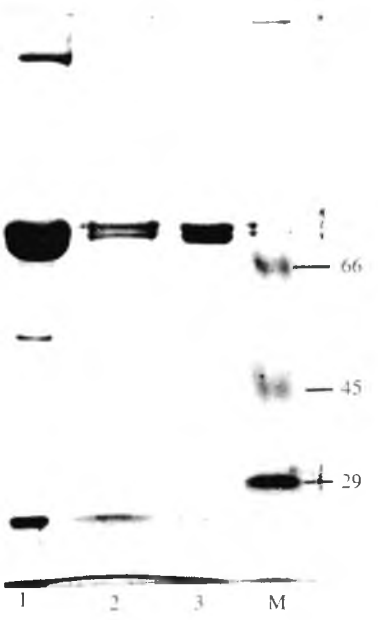


FIGURE 3. SDS-PAGE showing a comparative profile of proteins at various steps of purification from *C. cephalonica*. Lane 1: crude haemolymph, lane 2: 14th fraction eluate from Sephadex G 50–150 matrix, lane 3: pooled peak fraction eluate from DEAE Sephacel column and lane M: molecular weight marker (in kiloDaltons).

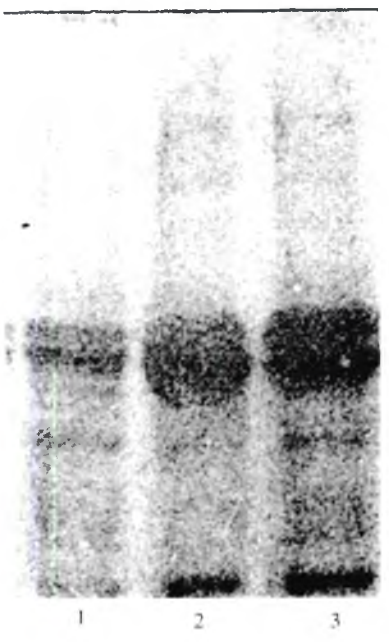


FIGURE 4. Immunoblot to show detection of SPs in crude haemolymph of different stages of *C. cephalonica* using antibodies raised against purified SPs obtained from the present protocol. Lane 1: early-last instar, lane 2: mid-last instar and lane 3: late-last instar larvae.

(c) Immunological studies

For this study the total haemolymph proteins from early, mid and late-last instar larvae were separated on Tris-glycine SDS-PAGE and electroblotted on nitrocellulose membrane. Proteins in the haemolymph were detected using antibodies raised against purified SPs (obtained from present protocol). The results presented in Fig. 4. showed the strong cross reactivity of antibodies with high molecular weight SPs present in the haemolymph at all stages of development.

Purification of SPs from *Bombyx mori* haemolymph

In order to devise a common protocol for the purification of SPs, the above mentioned protocol was also used for the purification of SPs from *B. mori* and the results are presented in Figs 5a–d. The peak fractions obtained from Sephadex G 50–150 column (Fig. 5a) revealed the presence of high as well as low molecular weight proteins on electrophoregram (Fig. 5b). The initial fractions of the peak (lanes 2 & 3) contained mainly high molecular weight protein, the middle fractions (lanes 4–6) showed the presence of high as well as low molecular weight proteins and the later fractions showed mainly the presence of only low molecular weight protein (lanes 7 & 8). When these peak fractions were pooled and passed on to the DEAE-Sephacel column two distinct peaks were obtained (Fig. 5c.). The first peak (Fig. 5d, lane 5) showed the

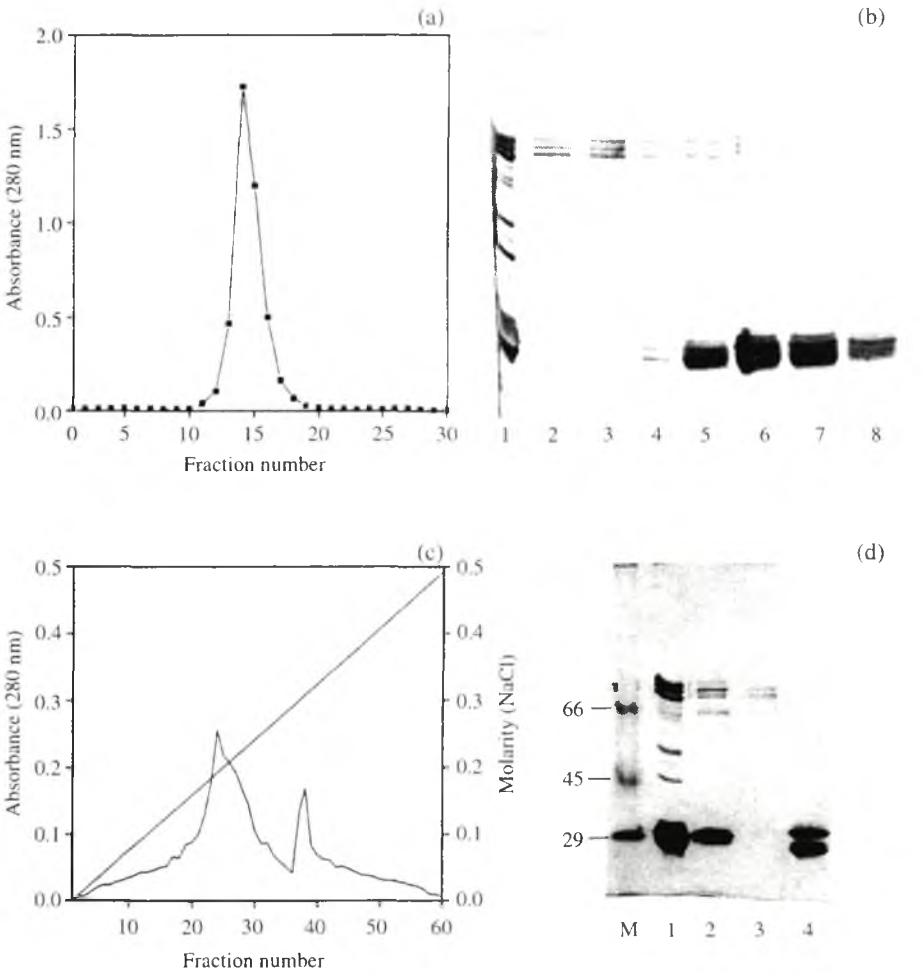


FIGURE 5. (a) Elution profile of haemolymph proteins of *B. mori* on Sephadex G 50–150 column; SDS-PAGE of peak fractions of *B. mori* haemolymph proteins eluted from Sephadex G 50–150 column. Lane 1: crude haemolymph, lanes 2–8: 12-18th fractions; (c) Fig. 5c. Elution profile of SPs of *B. mori* from DEAE Sephacel column using a linear gradient of 0–0.5 M NaCl: (d) SDS-PAGE showing a comparative profile of proteins at various steps of purification from *B. mori*. Lane M: molecular weight marker (in kiloDaltons), lane 1: crude haemolymph lane 2: 15th fraction eluate from Sephadex G 50–150 matrix, lane 3: 2nd peak, pooled fraction eluate from DEAE Sephacel column and lane 4: 1st peak, pooled fraction eluate from DEAE Sephacel column.

presence of only low molecular weight proteins and the second peak (Fig. 5d, lane 4) showed the presence of high molecular weight SPs. Table 1b represents the recovery of protein at each step of purification from *B. mori* haemolymph.

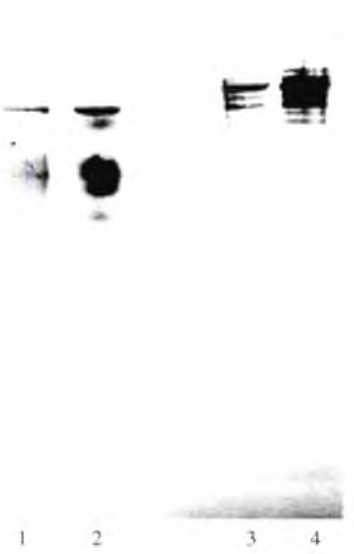


FIGURE 6. Native PAGE profile of *C. cephalonica* and *B. mori* SPs eluted from Sephadex G 50–150 column. Lanes 1 & 2: 15th & 16th fractions of *B. mori* obtained from haemolymph proteins using Sephadex G 50-150 column and lanes 3 & 4: 13th & 14th fraction of *C. cephalonica* obtained from haemolymph proteins using Sephadex G 50–150 column.

PAGE profile of *C. cephalonica* and *B. mori* SPs eluted from Sephadex G 50–150 column

To confirm the native molecular weight of SPs of *C. cephalonica* as well as *B. mori* and also to understand the nature of low molecular weight proteins of *B. mori* the fractions eluted from Sephadex G 50–150 matrix were subjected to 5% native PAGE (without SDS and reducing agent). The results presented in Fig. 6 revealed the presence of 450–500 kiloDalton native storage protein. Further more this study also suggested that the low molecular weight protein of *B. mori* is a subunit of the storage protein.

DISCUSSION

In the present study SPs have been purified by a two step purification protocol from the lepidopteran insect *C. cephalonica*. Result revealed the presence of storage protein in the larval haemolymph of *C. cephalonica* with a native mass of 450–500 kiloDalton. The purified SP produced three peptides on SDS-PAGE with mass of 86, 84 and 82 kiloDalton confirmed the earlier reports from our laboratory (Kirankumar *et al.*, 1997) that the SP in *C. cephalonica* is a hexamer composed of three subunits.

The devised protocol involves use of two columns, a gel filtration Sephadex G 50–150 column and an ion exchange DEAE Sephacel column. At gel filtration step of purification, almost 80% of the contaminants were eliminated and it also gave a good yield (approximately 60–65%) of SPs. The use of the DEAE Sephacel matrix further

purified the protein, which is evident from the presented result. The yield of SPs after this step was approximately 20–25%.

The same purification protocol was also extended to purify SPs from *B. mori*. Results presented clearly show that the devised protocol could be successfully used for the purification of SPs from the haemolymph of other lepidopteran insects. The major difference noticed in the purification of SPs of *B. mori* when compared with *C. cephalonica* was the difference in the optimal concentration of salts, which eluted the peak fraction. The other interesting difference noticed was that upon subjecting the pooled peak fractions eluate from Sephadex G 50–150 column on to a DEAE Sephacel column, the SPs got resolved into two distinct peaks showing the presence of the high as well as low molecular weight of storage protein subunits. The low molecular weight protein of *Bombyx mori* was identified as a subunit of storage protein by subjecting the Sephadex G 50–150 eluted fractions to native PAGE. This confirms the earlier study of Tojo *et al.* (1980) who reported the presence of 29 kiloDalton SP in *B. mori*.

The present protocol is a low cost method as it involves the use of only two matrices and routine laboratory chemicals. It is also rapid and the major advantage of this method is that it requires small amount of starting material (haemolymph proteins) and gives good yield of SPs with a high fold of purification. The high molecular weight of SPs, its abundance in larval haemolymph and its ability to bind to anion exchange matrix (Kirankumar *et al.*, 1997) was exploited in this protocol to facilitate the rapid purification.

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Biodiversity of Insects Trapped Over Bay of Bengal

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ABSTRACT: Wind borne insects of terrestrial origin in the aeolian environment over Bay of Bengal were trapped using multinet trapping system aboard the Ocean going Research Vessel (ORV), *Sagar Kanya*. Terrestrial insects that had fallen in the flotsam and the endemic ones (Halobatinae) were also collected using regular neuston nets. Wind borne insects trapped belonged to eleven orders of which four *viz*., Hemiptera (94%), Diptera (4%), Hymenoptera (1%) and Coleoptera (0.5%) accounted for almost 99% of the total haul of 16429 in two traps with three nets in each, operated continuously for the duration of the cruise. Trichoptera, Neuroptera, Thysanoptera, Odonata and Dictyoptera were represented by single digit numbers. Although order-wise diversity appeared to be low in this catch, diversity, with families and types in focus, was quite high. Out of a total of 92 families, 28 belonged to Diptera, 19 to Hymenoptera, only 16 to Hemiptera, 14 to Coleoptera and the rest to seven other orders represented by small numbers. Aerial density of these insects was not uniform and the sampling stations were grouped in clusters on the basis of the relative density of insects. Indices of species diversity, richness, and evenness highlight interesting aspects of this population. Homoptera dominated most of the clusters (Aphididae in clusters 1&2, Psyllidae in clusters 4 to 7, Delphacidae in clusters 8&9) except in cluster 3, which had Agromyzidae (Diptera) as the dominant group. A total of 83 insects including 23 live specimens of *Halobates* were recovered from the flotsam. These belonged to Hemiptera (82%), Diptera (4%), Coleoptera (4%), Hymenoptera (1%) and Neuroptera (1%). The rest (8%) could not be identified. Of the endemic insects, 21 specimens of *Halobates germanus* and two specimens of *Halobates micans* were trapped, both reported for the first time from this region. Contribution of insects to the oceanic biomass in the study area was found to be 45 g/km^2 including 13 g/km^2 contributed by *Halobates*. The standing crop was 8.2 kg/km^2/yr including a contribution of 5.9 kg km^2/yr by insects of terrestrial origin.

KEYWORDS: Insects of terrestrial origin, insect drift, Bay of Bengal, *Halobates germanus*, *Halobates micans*, oceanic biomass.

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†Deceased

INTRODUCTION

It has been surmised that large number of insects, mostly small, get caught in the convection currents on land and are then carried passively over and across the oceans by winds emanating from land of their origin (Cheng, 1976). Most of these insects however, fall in sea waters and either drown and disintegrate or get eaten up by plankton feeders within a period of 48 h (Cheng and Birch, 1977) and finally contribute to the oceanic biomass. The first systematic study on insect drift over oceans was that of Hardy and Milne (1937) who trapped airborne insects in the North Sea, with nets mounted on large kites, flown up to a height of 140 m above sea level from ships 200–300 km off the shore. Their trappings consisted mainly of small flies and aphids. An extensive programme of trapping insects over the Pacific ocean was undertaken by Gressit and his colleagues (Gressit *et al.*, 1961, 1962; Yoshimoto and Gressit, 1961; Yoshimoto *et al.*, 1962a,b; Harrell and Holzapfel, 1966; Holzapfel and Harrell, 1968). These workers collected insects in the Pacific, several hundred kilometers away from land. Aphids and small flies dominated their collections. Clagg (1966) studied the Atlantic–Antarctic area and related insects of the neighbouring continents to those trapped at sea and thus helped in visualizing a dispersal pattern (Bowden and Johnson, 1976). No such dispersal patterns have been worked out over Indian Ocean which has maritime continents known to be extremely rich in insect diversity and numbers. We collected data of wind borne insects of terrestrial origin in early summer over Bay of Bengal, which, combined with similar data in other seasons, could provide trans-oceanic dispersal patterns, if any, over this area. Other studies of ocean-going insects over Indian Ocean include those of Yoshimoto *et al.* (1962b) along the eastern coast of India, in the course of the Danish "Galathea" expedition of 1950–1952, Pathak and Parulekar (1988); Pathak (1998); Pathak *et al.* (1997, 1999a,b,c, 2000).

MATERIAL AND METHODS

Area of investigation

Almost the entire Bay of Bengal (between Latitude 4 to 21°N and Longitude 78 to 92°E was covered in the multidisciplinary cruise #100 (Fig. 1) of the Ocean going Research Vessel (ORV), *Sagar Kanya* from March 10 to April 9, 1995.

Trapping of airborne insects

Airborne insects were trapped with the help of a specially designed multinet trapping system (patent pending) for use on a moving vessel, as described earlier (Pathak *et al.*, 1999b). In the present case three nets, one above the other, were arranged on each side of the bridge of the vessel, over a height of 18 to 22 m above the sea surface. The nets could be hoisted up or lowered whenever required and could rotate freely so that their mouths (75 cm diam) always faced the wind. The nets remained hoisted 24 h every day for the duration of the cruise. Each net was lowered and examined for any insects trapped, twice every day, at 0730 and 1800 hrs, the locations referred as the sampling stations. The coordinates of these sampling stations and date and time

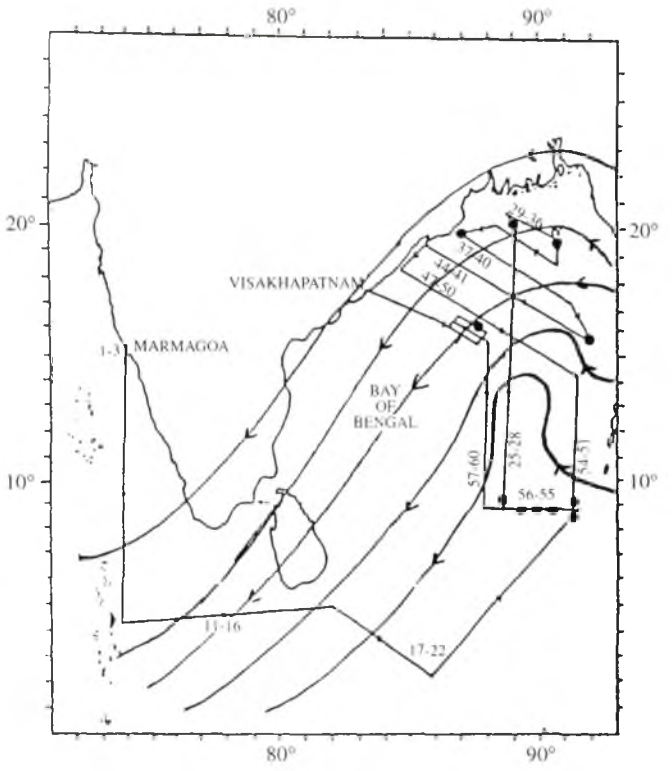

FIGURE 1. Track of cruise #100, ORV *Sagar kanya* in Bay of Bengal during March–April 1995. Numbers along the track indicate sampling stations for wind borne insects. Superimposed on the track is the generalized wind flow pattern during the period.

of removal of the insects from the nets were noted. Sampling stations were separated from one another by location and time but sometimes by time alone when the ship was stationary. The insects removed from the nets in the morning represented those trapped during the previous night while those removed in the evening represented day trappings. The speed of the vessel varied from time to time as per requirements of other experiments being conducted on the vessel during the cruise. Insects were sorted and immediately transferred to glass vials containing ethyl alcohol (80%). Identification (mostly to families, but in some cases to genera and species) was carried out later in the laboratory. Although identification was not carried out to species in all cases, it was confirmed that there were 215 recognizable taxonomic units, on the basis of morphological differences.

Trapping of insects in the flotsam

Both the airborne insects that had fallen in water as well as those endemic in the open ocean were collected using standard neuston nets which were towed for 20 min at a

time with ship speed maintained between 4–5 knots per hour. Any insects/insect parts, present in the collected material were carefully sorted out in the laboratory.

Meteorological data

Wind direction was noted with the help of the wind vanes mounted on the bridge, at 0730, 1400 and 1800 hrs. The readings were then corrected in relation to the direction of the ship movement with the help of standard tables (Marine Observer's Handbook, 1992). Wind speed was measured with portable anemometers (OGAWA SEIKI, Japan) and corrected for the ship speed and direction of movement using standard tables. Generalized wind flow patterns (Fig. 1) were then drawn using these readings and the records of wind direction and speed in the study area, provided by the national data centre for the Indian Ocean, Goa. The distance of the nearest land mass along the generalized wind flow worked out, was then estimated from a map of the area drawn to scale. Such a definition of the nearest land mass became necessary as the insects were virtually carried downwind by the wind currents.

Calculation of the aerial density of insects

Aerial density of insects over the ocean was calculated by dividing the number of insects trapped by the product of the distance travelled, height (4 m i.e. between 18 and 22 m) and width (1.5 m—the diameter of the net opening being 0.75 m on each side) and expressed as number of insects/km^3 at a height of 18 to 22 m above sea surface. For these calculations the wind speed was assumed as zero. Contiguous stations with similar level of trapped insects, together formed one cluster.

Analysis of data

The data generated were subjected to the following analyses:

1. Frequency distribution was studied by plotting the number of species against the number of individuals per species, after Warwick and Buchanan (1970). The frequency was plotted on arithmetic scale, the vertical lines indicating the observed distribution and the crosses joined by a broken line showing the theoretical log series distribution.
2. α-Index of diversity was calculated after Williams (1964) and as described earlier (Pathak *et al.*, 1999b). α is a constant and has been found to be a measure of the density of the population.
3. Relationship between α-index of diversity and the distance of the nearest land mass *in wind direction* by plotting one against the other.
4. Correlation between insect size and the distance by a scatter plot of insect size and the distance of the nearest land mass *in wind direction*.
5. Indices of species diversity, richness and evenness were calculated after Shannon and Weaver (1963).
6. Insect contribution to the oceanic biomass was calculated after Cheng and Birch (1977).

TABLE 1. Airborne insects trapped over Bay of Bengal aboard ORV *Sagar Kanya*, March–April 1995

Orders	Number of Families	Species	Specimens
Hemiptera	16(17.4)	45(20)	15 454(94.6)
Diptera	28(30.4)	86(40)	611(3.7)
Hymenoptera	19(20.6)	46(21.4)	199(1.2)
Coleoptera	14(15.2)	22(10.2)	83(0.5)
Trichoptera	05	07	24
Neuroptera	02	03	17
Odonata	01	01	11
Orthoptera	01	01	04
Thysanoptera	02	02	03
Dictyoptera	01	01	01
Lepidoptera	03??	??	22
Total	92	215+	16429

Figures in parentheses represent per cent of the total catch.

RESULTS

Wind-borne insects trapped

A total of 16429 insects were trapped at 51 sampling stations. These belonged to 11 orders, 92 families and 215 species (Table 1). Figure 2 shows the proportion of families, species and individuals, of the total catch. All, except for less than six per cent of the total specimens, belonged to Hemiptera. Diptera were the next in numbers while Hymenoptera was the third most numerous order. Small beetles constituted the fourth group. Trichoptera, Lepidoptera, Neuroptera, Odonata, Orthoptera, Thysanoptera and Dictyoptera registered only a presence with contributions less than 0.5%.

The trappings reflect that although Hemiptera had the largest number of specimens in the catches, Diptera was the most diverse order in terms of the number of families as well as species.

Out of the 92 families trapped, Chloropidae (Diptera: Cyclorrhapha) had the highest number of species (13). Jassidae and Phoridae were the next two with 11 and 10 species, respectively. Cecidomyiidae and Agromyzidae had eight species each while Delphacidae and Clusiidae were represented by 7 species each. Psyllidae and Aphididae had six species each. Three families *viz.*, Eurytomidae, Platygasteridae and Miridae had five species each. Four families *viz.*, Agaontidae, Braconidae, Formicidae and Bibionidae were represented by four species each. Eight families had three species each while there were as many as sixteen families with two species each. The diversity was apparently high as seen by a very large number of families (49) that were represented by a single species each. It may however, be mentioned that the three families and species of Lepidoptera (microlepidopterans) remained unidentified.

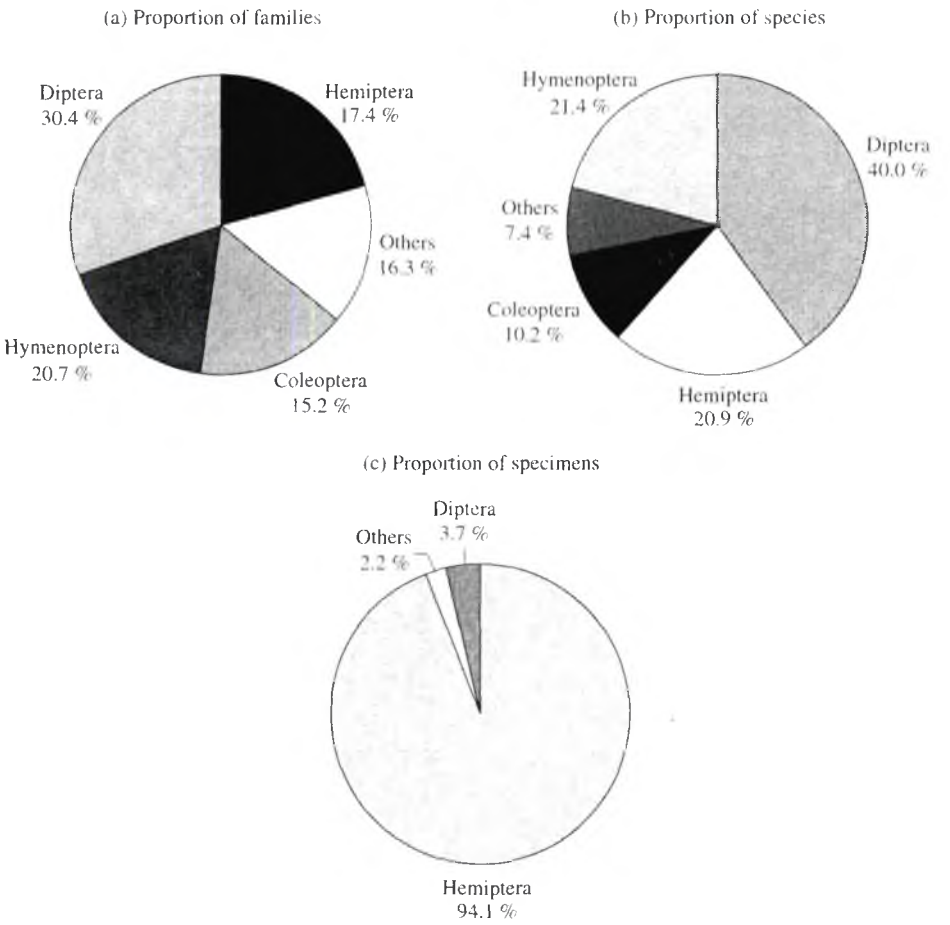

FIGURE 2. Pie chart showing the proportion of (a) families, (b) species and (c) individuals belonging to various insect orders in the trappings of wind borne insects over Bay of Bengal.

Aerial density of insects over the area of investigation

A comparison of the number of insects trapped and the area covered, in a span of 4 m (between 18 to 22 m above sea surface) gave us the aerial density of these insects. But the density was not uniform all over, being very high in some areas and very low in others (Fig. 3). There were nine clusters in all, with the density varying very widely (Fig. 3, Table 2). It may be noted that much overlapping was seen between various clusters. But although these clusters overlapped in dimensions, they were separated by time. Apparently the varying density of insects of terrestrial origin in these clusters depended upon their density on the land of their origin at the time of their being caught in the wind currents.

TABLE 2. Analysis of the trapping of insects of terrestrial origin over Bay of Bengal. ORV *Sagar Kanya*, March–April 1995

Clusters, with av. distance *in wind direction* in km (in parentheses)	Aerial density in $n \times 10^4$/ km^3	Total number of individuals of all species (N)	Species Number (S)	Diversity (H)	Evenness (J)	Dominant Family (*P* max)
1(830)	28.20	110	15	1.96	0.72	Aphididae
2(870)	1.90	53	17	2.33	0.82	-do-
3(450)	1.21	9	4	1.00	0.72	Agromyzidae
4(350)	76.36	2588	78	1.74	0.40	Psyllidae
5(240)	48.92	69	16	1.99	0.72	-do-
6(180)	1267.88	9435	115	0.67	1.14	-do-
7(300)	6.50	72	10	1.20	0.52	-do-
8(200)	272.51	4073	62	1.55	0.38	Delphacidae
9(270)	1.31	21	8	1.49	0.72	-do-

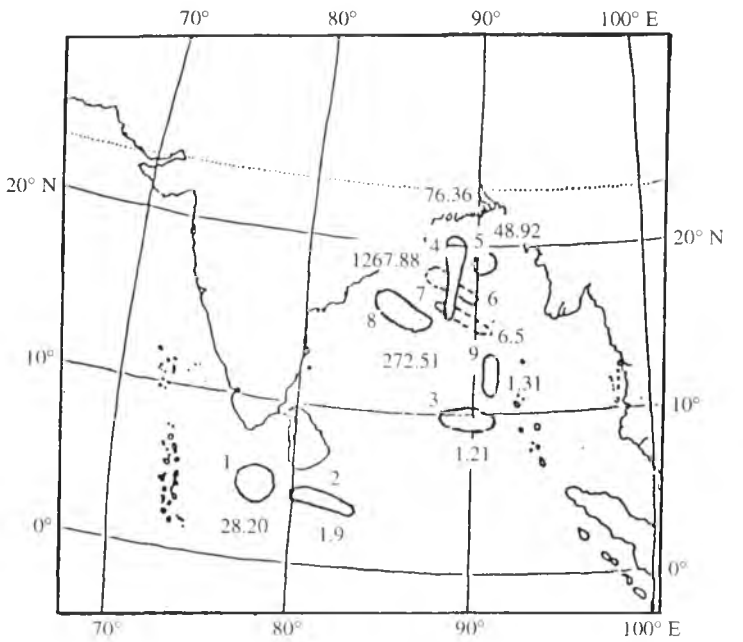

FIGURE 3. Aerial density (per km^3) of insects in different clusters of the study area in Bay of Bengal.

Indices of species diversity, richness and evenness

Table 2 presents various indices and the dominant groups in the nine clusters. The species diversity index is the highest in those clusters that are the farthest from land

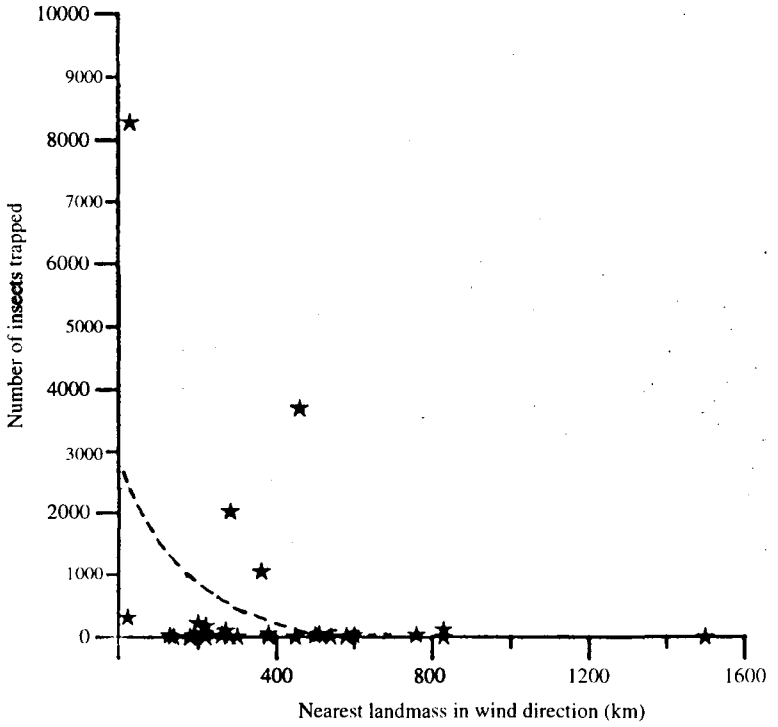

FIGURE 4. Curve showing the relation between the number of insects trapped in Bay of Bengal and the distance of the nearest landmass *along the wind direction*. The best fit for the curve was $\log(Y) = B(X) + A$.

(clusters 2 and 1) and the lowest in a cluster that is closest to land (cluster 6). The number of species detected in these clusters varied from a low (4) in cluster 3 to a high (115) in cluster 6. Except for one cluster (3) in which Agromyzidae was the dominant family, all other clusters were dominated by three families belonging to Homoptera.

Correlation between distance, diversity, abundance and size of the insects

There was a negative correlation (Pearson's $r = -0.249$; $p = 0.05$) between distance of the nearest land mass *in wind direction* and the number of insects trapped, the number declining with increase of the distance (Fig. 4). There were hardly any insects in clusters #1, 3 and 7. The index touched a high of 23 in cluster 6 and fluctuated between these extremes in other clusters. The α-diversity index varied similarly and showed a negative correlation which was non significant (Pearson's $r = -0.541$; $p = 0.05$). Frequency and log series distribution are shown in Fig. 5. Relation between insect size and the distance of the nearest land mass *in wind direction* (Fig. 6)

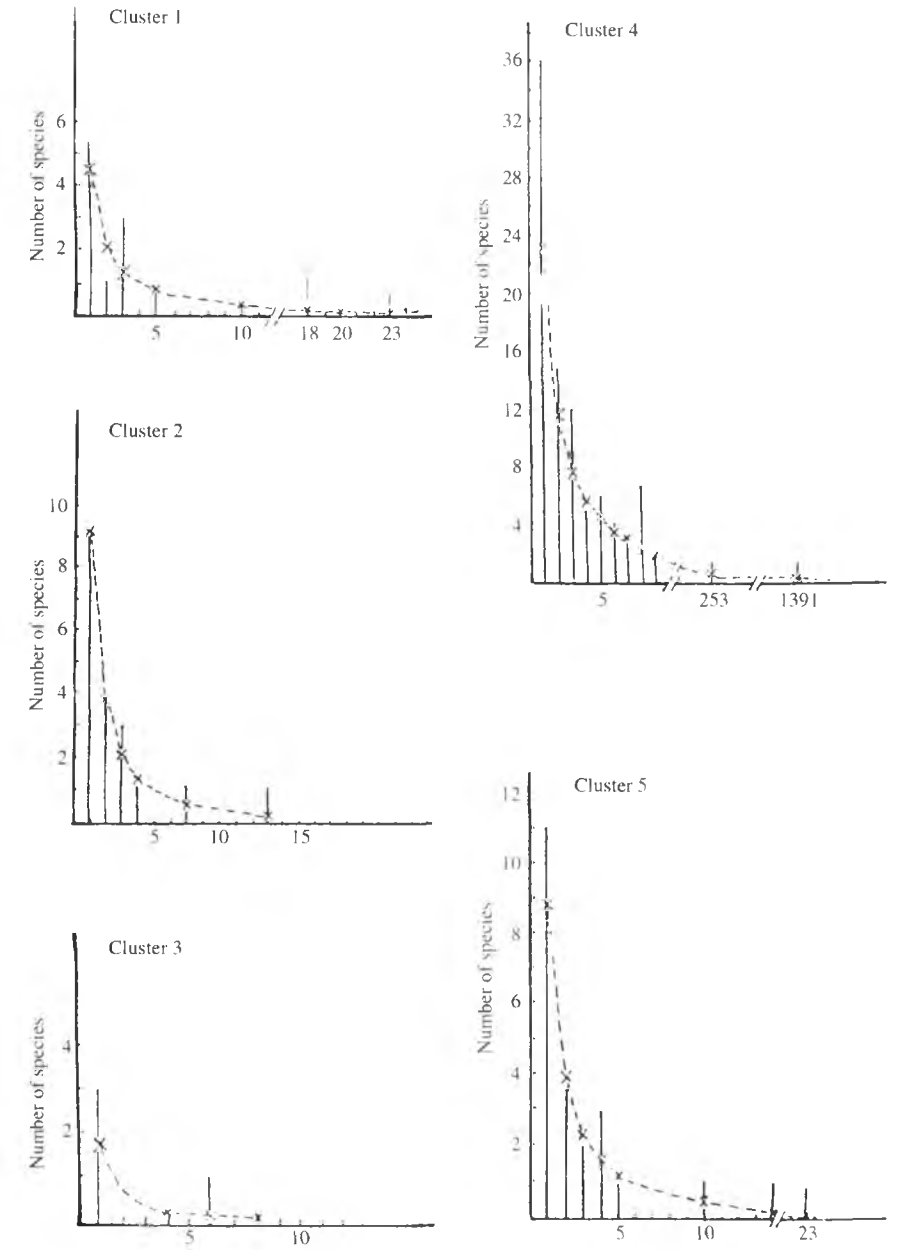

FIGURE 5. Frequency distribution of the insects in clusters 1 to 9. The frequency was plotted on an arithmetic scale, vertical lines indicating the observed distribution (of number of individuals per species) and the crosses joined by a broken line showing the theoretical log series distribution.

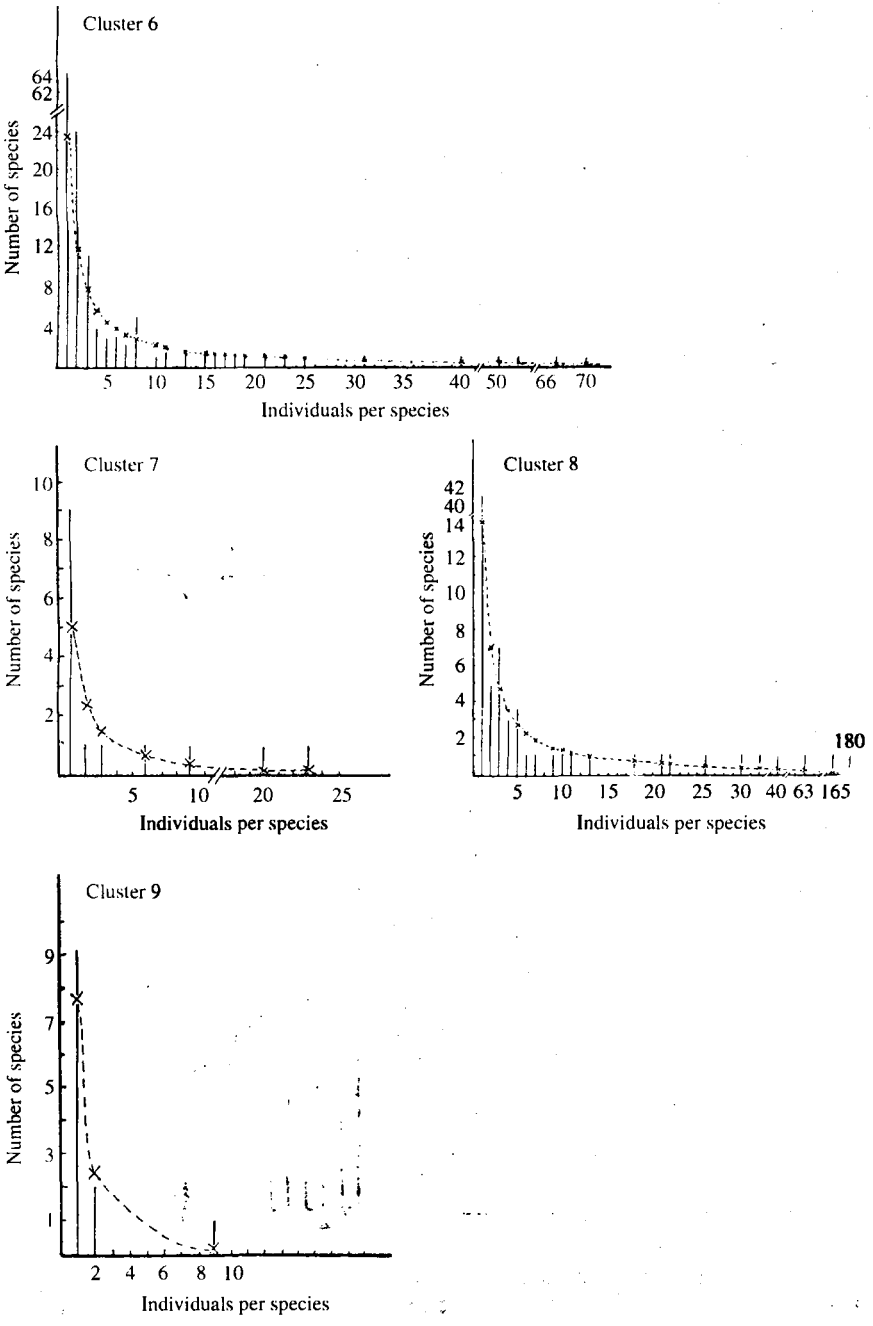

FIGURE 5. Continued.

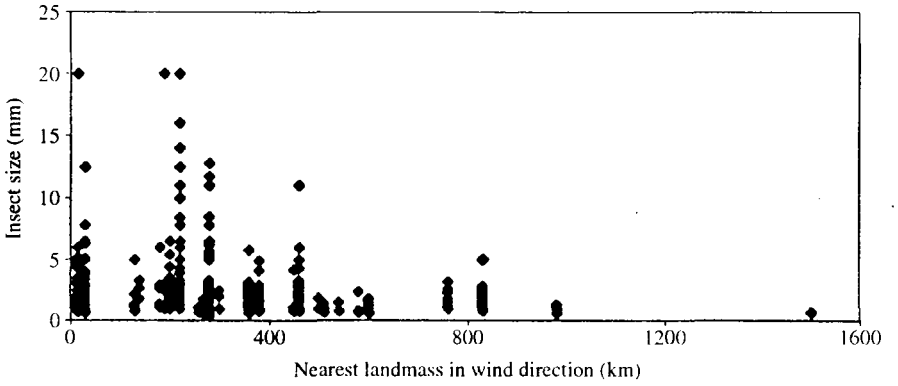

FIGURE 6. Scatter plot showing the relation of insect size and the distance of the nearest land mass *in wind direction* (km) in Bay of Bengal.

TABLE 3. Insects of terrestrial origin, in the flotsam in Bay of Bengal Cruise no. 100, ORV *Sagar Kanya*, March–April 1995

Order	Number of Families	Insects Trapped	
		number	percent
Hemiptera	3	44	80.72
Diptera	2	4	4.82
Hymenoptera	1	1	1.20
Coleoptera	2	3	3.61
Neuroptera	1	1	1.20
Unidentified		7	8.43
Total	9	60	

Notes:
(1) Besides the above, 23 insects, endemic in the sea water, were also recovered, taking the total to 83; (2) Number of tows (20 min each): 11; (3) Area covered per tow: 1848 m^2; (4) Number of insects per km^3: 0.45×10^5; (5) Number of insects of terrestrial origin in the flotsam: 0.32×10^5; (6) Total insect contribution to the oceanic biomass: 45 g/km^2; (7) Contribution of insects of terrestrial origin: 32 g/km^2; (8) Total standing crop of insects in Bay of Bengal: 8.2 kg/km^2/yr; (9) Standing crop of Insects of terrestrial origin; in Bay of Bengal: 5.92 kg/km^2/yr.

was indicated by a negative correlation, ($r = -0.10065$), though statistically non-significant.

Insects recovered from the flotsam

A total of 83 insects were recovered from flotsam samples in the area (Table 3). These included 60 dead insects of terrestrial origin besides 23 endemic ones belonging to subfamily Halobatinae. In addition, a number of disintegrating insect parts, were also present in the sample. Of the 60 dead insects of terrestrial origin, seven remained

unidentified, while 44 belonged to Hemiptera (mostly Homoptera), 4 to Diptera, 3 to Coleoptera and one each to Hymenoptera and Neuroptera. This came to almost 0.45×10^5 insects per km^2. Of the 23 live specimens 21 belonged to the species *Halobates germanus* while two belonged to *H. micans*. Assuming average weight of an insect to be 1 mg, the contribution of all these insects to the oceanic biomass was found to be over 45 g/km^2. Three–fourth of this contribution was that of insects of terrestrial origin. The standing crop of insects in the Bay of Bengal was calculated to be over 8.2 $kg/km^2/yr$, out of which the contribution of insects of terrestrial origin was 5.9 $kg/km^2/yr$.

DISCUSSION

There have been only a few reports, on the dispersal of air borne insects from various parts of the Indian Ocean. Our observations in the northern part of the Arabian Sea in early summer (Pathak *et al.*, 1999b) revealed Hymenoptera as the most numerous order as to the number of specimens, with Hemiptera, Diptera and Coleoptera following, in decreasing order. Neuroptera, Trichoptera, Lepidoptera and Orthoptera just marked their presence with less than 1% of specimens in each case. In the present investigation however, Hemiptera (two third of which were homopterans) predominated with Diptera and Hymenoptera relegated to the second and third positions, respectively. Coleoptera continued to be at fourth position. Neuroptera, Trichoptera, Lepidoptera and Orthoptera just marked their presence. If we compare these data with those from other areas like the North Sea (Hardy and Milne, 1937) and the Pacific Ocean (Harrell and Holzapfel, 1966), we find that Homoptera, Diptera and Hymenoptera are the three dominant orders with Coleoptera a distant fourth even in these areas. Preponderance of a particular group in the trappings represents higher occurrence of that particular group in a particular season, on the land from which the winds originated. This statement however needs to be qualified with two factors. Firstly larger (heavier) insects which may have been present on the land mass in question would mostly be sorted against and left behind while the smaller ones carried out over the ocean. Secondly the composition of an insect population over the ocean would also depend upon insects that were 'in flight' at the time of being caught in the thermal currents or seaward winds. Comparison of these data with those in the Pacific would not be very relevant, but it is found that the dominant orders in various collections in the Pacific area are also the same as seen in the present work, these orders being Hemiptera, Diptera, Hymenoptera and Coleoptera. While Coleoptera was always at a fourth position, other three orders changed positions in various trappings. This is also true with regard to other areas of the Indian Ocean *viz.*, north Arabian Sea and Indian Ocean south of the Indian peninsula (Pathak, 1998; Pathak *et al.*, 1997, 1999b, 2000). Dominance in various clusters varied in the three sectors of study in the Indian Ocean. While Homoptera dominated in 8 out of 9 clusters in the present study in Bay of Bengal, it was Agaontidae (Hymenoptera) that dominated in four out of five clusters in north Arabian Sea. In Indian Ocean (south of the Indian peninsula), four out of six clusters had homopteran families (*viz.*, Fulgoridae, Jassidae

and Chermidae) dominating while Cecidomyiidae (Diptera) and Agaontidae were the dominant families in the other two.

The possibility of there being more insects, migratory in nature, in these populations over the ocean was checked. It was found that aphids and planthoppers, which are such migratory insects, constitued only less than half of the total homopterans which in turn were less than one third of the total insects trapped.

Family wise distribution was generally similar to other collections over other oceans, but differed in one aspect. Hardy and Milne (1937) had pointed out that Acalyptrate dipterans (represented by Chloropidae, Drosophilidae and Agromyzidae) formed as much as 69% of the total dipterans, in their collection over the North Sea. In the present case however, Acalyptrates form only 13.4% of the Diptera trapped. A comparison of families present in the north Arabian Sea, Indian Ocean and the Bay of Bengal showed there were 26 families common to the three regions. Prominent among these were Leptoceridae (Trichoptera), Jassidae and Fulgoridae (Homoptera), Miridae, Lygaeidae and Coreidae (Heteroptera), Chrysomelidae, Curculionidae and Carabidae (Coleoptera), Encyrtidae, Formicidae, Agaontidae and Braconidae (Hymenoptera), Cecidomyiidae, Chironomidae, Phoridae and Muscidae (Diptera). There were five families common to Bay of Bengal and Arabian Sea; these included Hydroptilidae (Trichoptera), Berytidae (Heteroptera), Erotylidae (Coleoptera) and Anthomyiidae (Diptera). The Bay of Bengal and the Indian Ocean had 13 families common to the two sectors. These included Chrysopidae, Aphididae, Anthocoridae and Saldidae, Scolytidae, Ichneumonidae and Incubidae, Culicidae, Tipulidae and Drosophilidae. There were as many as 48 families which were present only in the Bay of Bengal sector. Prominent among these were Listidae (Odonata), Limnephilidae and Sericostomatidae (Trichoptera), Coniopterygidae (Neuroptera), Thripidae (Thysanoptera), Psyllidae and Delphacidae (Homoptera), Gerridae, Mesoveliidae and Pentatomidae (Heteroptera), Dytiscidae, Coccinellidae, Staphylinidae and Bruchidae (Coleoptera), Colletidae, Siricidae and Torymidae (Hymenoptera), Bibionidae, Blepharoceridae, Dixidae, Asilidae, Tephritidae, Syrphidae, Glossinidae, Conopidae, Hippoboscidae and Otitidae (Diptera).

It is not known whether there is any seasonal variation in the number and variety of trapped insects. It is logical to expect a seasonal variation, directly linked to the direction of the wind flow in various seasons and the land mass(es) from where these winds, carrying insects, originate. But such repeat trappings in various seasons have not been carried out anywhere in the Indian Ocean.

Trappings of insects have been almost equal during the days and nights. This was as expected because occurrence of insects at a particular location over the sea at a particular time depends upon the time when the winds started moving seawards and the direction and speed of this movement. The time of arrival of the insects at a particular location could thus be any time during 24 hours.

It is notable that small insects (0.4 to 3 mm in length) dominate the present collection. This is similar to most other observations in the Pacific and other oceans over which airborne insects have been trapped (Bowden and Johnson, 1976). Most of

these insects are weak fliers and their dispersal appears to be almost entirely passive in nature. Wind patterns have helped workers to determine the possible land of origin of the insects trapped at sea (Holzapfel and Harrell, 1968). In the present investigation, no definite suggestion has been made regarding the possible land of origin of the trapped insects. Although, the wind flow pattern does provide a general idea of the origin of these insects, other relevant information like the aerial density of specific insect species over various land masses, in various seasons in the region, is not available.

A negative correlation does exist betwen the distance of the nearest land mass *in wind direction* and the number of insects trapped, the number of airborne insects over the ocean apparently decreasing with increasing distance.

Flotsam continues to be an 'unstudied' resource of the sea (Cheng and Birch, 1978). There are only a few reports about the insect contribution to the oceanic biomass (Zaitsev, 1970; Cheng, 1975; Bowden and Johnson, 1976; Cheng and Birch, 1977, 1978). Compared to the data provided by these authors in Black Sea, North Pacific, North Sea, Gulf of California, and English channel the contribution in the Bay of Bengal is only slightly lesser. While Hemiptera dominated the insects of terrestrial origin in Bay of Bengal, the dominant family in Gulf of California was Cicadellidae, while Staphylinidae and Chironomidae dominated the collection in the English channel (Cheng and Birch, 1978).

There have been a number of reports of *Halobates* from various areas of the Indian Ocean. Cheng (1985) and Andersen and Foster (1992) described various species of this genus in the coastal regions of India. The latter authors described five species including a new species *H. elephanta*. Two species *viz.*, *H. germanus* and *H. micans* have been reported from various sea waters. *H. micans* appears to be more common in the western part of the Indian Ocean (Cheng, 1973), and had never been noticed in the eastern sector or Bay of Bengal. *H. germanus* which also dominates the Pacific, has been reported from Nosy Be, Magalasy (Cheng, 1974) and Aldabra and other nearby atolls north of Madagascar (Polhemus and Polhemus, 1991), although the occurrence of this species west of South China Sea is rather rare. It is for the first time that *H. germanus* and *H. micans* have been collected from the Bay of Bengal.

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Ultrastructural Details of the Morphological Adaptations of Some Tea Pests of Assam (India)

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ABSTRACT: A Scanning Electron Microscopic study of some of the major/minor tea pests (*viz.*, Live-wood eating termite, *Microtermes obesi*, Holmgreen; Scavenging termite, *Odontotermes* sp; Red spider mite, *Oligonychus coffeae*, Nietner; Thrips, *Scirtothrips dorsalis*, Hood and tea Aphid, *Toxoptera aurantii*, Boyer) of Barak Valley, Assam was undertaken. Emphasis was laid on the ultrastructural studies on cuticle and its associated sensilla, mouth parts, sucking organs, locomotary organs etc. The serrated mandible is the distinguishing feature of soldier of *M. obesi*, which is absent in *Odontotermes* sp. This is related to the feeding behaviour of the pest. The well developed sensilla chaetica and sensilla basiconica in the body/appendages of *O. coffeae* suggests their role in chemoreception and reception of volatile chemicals from tea plants. The wavy striations of alternate electron dense and electroluscent bands act as anti-reflection device in *O. coffeae*. An anchor like structure at the tip of the leg of *S. dorsalis* is an important morphological adaptation. The bluntness of the tip of sucking organ of *T. aurantii* suggests their role in laceration of the tender shoots of tea plants. The role of different types of sensilla in the body surface/appendages of the above pests is discussed with respect to their functional significance.

KEYWORDS: Electron dense band, electroluscent band, sensilla, serrated mandible, tea pests, ultrastructural study.

INTRODUCTION

Tea plantation requires hot and humid climatic conditions. From the climatic point of view, tea is essentially a crop of the tropical region, but grows well in subtropical regions too. There seems to be no upper limit of rainfall as the growth of tea is concerned. However, the lower limit seems to be 127 cm. provided other environmental conditions exert mitigating circumstances (Baruah, 1989; Rustagi, 1995).

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Since the growth of tea requires unique climatic conditions, the insects and other arthropod pests attacking different parts of the tea plant must thrive in these specific environmental conditions. Therefore, it is quite certain that the pest would exhibit some specialisation in their morphological features, although significant modifications in gross morphology may not be expected. Detailed studies on the fine structure of various organs of the pest may exhibit some important modifications.

Keeping the above in view, a Scanning Electron Microscopic study on the mouth parts, antennae, general body cuticle, thoracic appendages, head and its appendages etc. of some of the major/minor tea pests of Barak Valley (Assam) was undertaken. Emphasis was laid on the ultrastructural studies on cuticle and its associated sensilla, since, success of insects as terrestrial animals in resisting desiccation, and, in breathing atmospheric air etc. are directly related to the properties of their integument (Smith, 1968). Once the structural specializations of the pests in relation to the host plant and prevailing environmental conditions are established, a well planned strategy can be adopted for the management of pests, which cause major economic losses to the tea industry at large.

MATERIAL AND METHODS

The predominant pests of Barak Valley (Assam) taken for the present study include (i) Live-wood eating termite (*Microtermes obesi,* Holmgreen), (ii) Scavenging termite (*Odontotermes* sp.), (iii) Red spider mite (*Oligonychus coffeae*, Nietner) and (iv) Thrips (*Scirtothrips dorsalis*, Hood), while the minor pest studied was the Aphid (*Toxoptera aurantii,* Boyer).

The parts used for ultrastructural studies were mouth parts, antennae, legs, head, dorsal and ventral body surface, cuticular folds and sensillae associated with them.

The parts excised from the body following exposure to benzene vapour were fixed in 2.5–3% glutaraldehyde prepared in 0.1 M sodium cacodylate buffer for 4 hours at 4 °C. The primary fixation in glutardehyde was followed by buffer wash of the samples for 20–30 minutes, and secondary fixation in 1% buffered osmium tetroxide for 30 minutes to one hour. The fixed samples were dehydrated through increasing concentrations of acetone and were dried either in critical point drier (CPD) using acetone as the intermediate fluid and carbon-di-oxide as the transitional fluid, or it was done with tetramethyl silane drying technique of Dey *et al.* (1989).

Observations were made with a Scanning Electron Microscope [JSM 35 CF (Jeol)] using the secondary electron emission mode. The accelerating voltage applied was 20 KV. The working distance (WD) selector was set at 15 mm, and the tilt control was fixed at zero degrees for setting the specimen stage in a horizontal position.

RESULTS

The SEM study of different body parts of the tea pests shows certain interesting ultra structural features that are relevant to the ecophysiology of the pests.

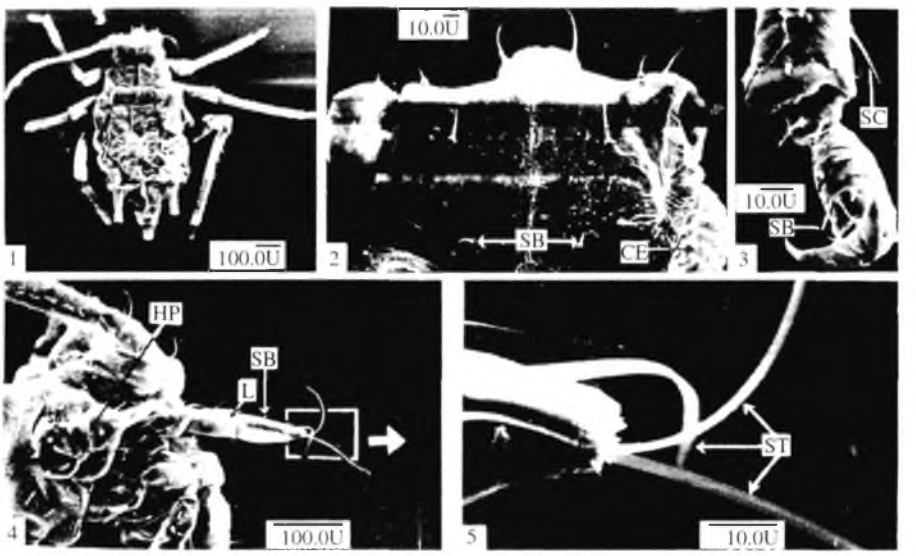

PLATE 1. Aphid (*Toxoptera aurantii*, Boyer) (1) Entire pest (dorsal view) (2) Anterior part of head showing sensilla basiconica (SB) and compound eye (CE) (3) Tip of leg showing sensilla chaetica (SC) and sensilla basiconica (SB) (4) Ventral view of mouth parts showing sucking organ, labium (L), sensilla basiconica (SB) on it and hypopharynx (HP) (5) The anterior part of the sucking organ (or Labium) showing three well developed sensilla trichodea (ST).

Aphid (*Toxoptera aurantii*, Boyer)

The most significant observations on ultrastructural features of aphid is the modification in hypopharynx and labium. The hypopharynx is characterised by the presence of swollen base and pointed tip. Beside these, it has some small sensilla with swollen base, probably sensilla basiconica with gustatory or chemoreceptive role. The labium, as expected is long (270 μm) and is modified for sucking the sap. However, unlike piercing sucking type of mouth parts, its tip is blunt. The labium shows three major segments about 70–120 μm long and 23–42 μm broad. Of these segments, the lowest one is the largest (120 μm) (Plate 1(4) L). The labium contains a few sensilla basiconica around 20–25 μm long and 2–3 μm broad with well developed sockets at its dorsal surface (Plate 1(4) SB). In addition, it contains some long (105–110 μm) sensilla trichodea at the tip, dorsal and dorso-lateral surface of the labium (Plate 1(5) ST). The lateral position of the head contains two well developed compound eyes with compact ommatidia (Plate 1(2) CE).

The legs of aphid are covered with sensilla chaetica, which probably acts as contact chemoreceptors (Plate 1(3) SC). As far as the gross morphology is concerned, the claws on the tarsus of pro, meso and metathoracic legs are bent inwards and are pointed and sharp, suitable for damaging/cutting plant parts and clinging to it (Plate 1(3)). Besides sensilla chaetica, the dorsal and dorsolateral surface of tarsus contains some

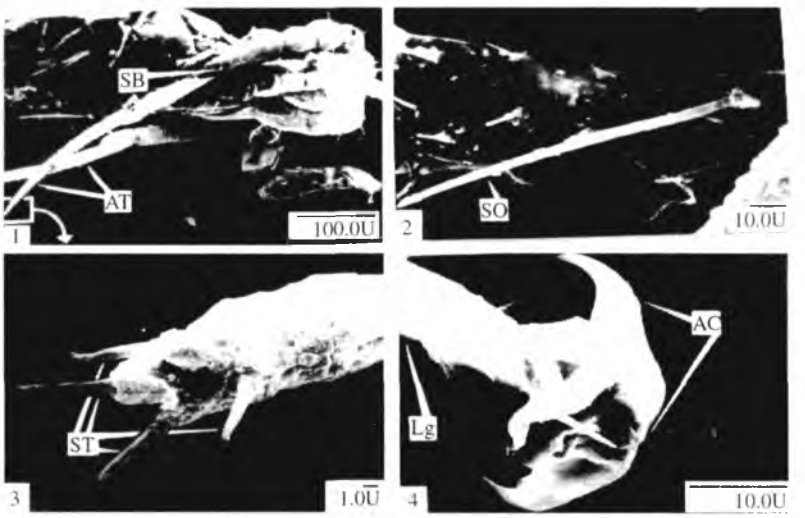

PLATE 2. Thrips (*Scirtothrips dorsalis*, Hood) (1) Mouth parts showing antennae (AT) and sensilla basiconica (SB) on it and the long sucking organ encompassed between two antennae (2) The tip and swollen base of the sucking organ (SO) (3) Tip of one antenna showing four well developed sensilla trichdea (ST) (4) The tip of leg (Lg) showing anchor like structures (AC).

short sensilla basiconica (3–5 μm) with well developed sockets (Plate 1(3) SB). The general body cuticle contains a few sensilla basiconica (20–40 μm long) with characteristic swollen base (Plate 1(2) SB).

Thrips (*Scirtothrips dorsalis*, Hood)

In thrips, antennae show some interesting features. The number and type of sensilla in antennae are few. The general morphology of the antennae, with a large number of cuticular folds suggests a highly flexible nature of the structure. The sensillae present on the antennae are mostly basiconic sensilla with characteristic features (i.e. swollen base) of chemoreceptors (Plate 2(1) SB). The most significant modification, however, is observed in the piercing-sucking mouth parts. The tip of the sucking organ is highly elongaged (96 μm) and the base is swollen (10 μm) (Plate 2(2) SO).

The tip of antennae show a number of sensilla trichodea (6.5–7 μm long and 1 μm broad) with striated surface. The base of the sensilla was found to be non flexible (Plate 2(3) ST). The tip of leg shows some anchor like structure (Plate 2(4) AC) being broad at the centre and pointed at the lateral part.

Red spider mite (*Oligonychus coffeae*, Nietner)

Scanning Electron Microscopy revealed detailed ultrastructural features of the red spider mite. The length and breadth or the body has been found to be around 350 μm and 170 μm respectively (Plate 3(1)). The appendages are well developed and are found to be equipped with a large number of sensilla chaetica and sensilla trichodea

PLATE 3. Red spider mite (*Oligonychus coffeae*, Nietner) (1) Entire organism in dorsal view (2) Cuticular fold and sensilla on dorsal body surface (3) Cuticulr fold on the body in enlarged view showing wavy striations of alternate electron dense (EDB) and electroluscent (ELB) bands (4) Cuticular fold in the head appandages showing sensilla trichodea (ST) and sensilla chaetica (SC) (5) Enlarge view of cuticular fold in head appendages showing alternate electron dense and electroluscent bands (6) and (7) Middle part and tip of a sensilla chaetica showing spiny outgrowths (SO) (8) Sensilla trichodea showing the socketed base(S) and longitudinal striations (LS) (9) and (10) Rammer shaped (R) sensilla chactica (SC) at the tip of leg.

(Plate 3(4) SC, ST). The general body cuticle shows the characteristic foldings observed in most of the mite species (Plate 3(3) EDB, ELB). The electroluscent bands are conical in appearance and are raised from the general cuticular plane. The height and periodicity of these conical structures are more or less uniform (0.5 μm and 1.5–2.2 μm respectively). The appendages also contain striations but unlike body surface they do not contain any conical structure (Plate 3(5)). The sensilla present on the surface of the appendages comprise mostly of sensilla chaetica about 70 μm in length and 2 μm in breadth. Besides, sensilla trichodea also occur in few numbers (Plate 3(4) SC, ST). The surface of the sensilla trichodea shows longitudinal striations (LS) and secondary ridges, (Plate 3(8)) while sensilla chaetica shows lateral spiny outgrowth (Plate 3(6), (7) SO). The tip of the sensilla chaetica is about 0.1 μm in breadth and is blunt (Plate 3(7)).

Sensilla trichodea present on the surface of appendages are found to be characterised by the presence of a well developed socket (2.8–3 μm in diameter)

(Plate 3(8) S) and longitudinal striations (Plate 3(8) LS), indicating their porous nature. The base of the sensilla has been found to be non flexible (Plate 3(8)).

The apical portion of the appendages has been found to contain about four highly specialised sensilla chaetica with some unique features (Plate 3(9), (10) SC). All these specialized sensilla appears to have a common origin at the apex of the appendages. The base of the sensilla is swollen, which gradually tapers, but again broadens at the tip. The broad tip has been found to be rammer shaped (Plate 3(10) R). All these sensilla have been found to be more or less of the same length (about 10.5–12.5 μm). The width of the swollen base, narrow central part and the flattened tip has been observed to be about 1.4, 0.4 and 7 μm respectively (Plate 3(9), (10).)

Live-wood eating termite (*Microtermes obesi*, Holmgreen)

Soldier

The dorsal surface of the head, thorax, abdomen and the thoracic appendages are found to be covered with cuticular spines ranging from 20 to 100 μm in length. These spines do not show the presence of a well developed socket and also do not exhibit the characteristic features of the chemoreceptors (Plate 4(1)). The ventral surface of the same region exhibits the similar type of sensory structure.

The significant feature of the mouth parts is the presence of serrated mandibles. The serrations has been observed at the inner edge (Plate 4(3), (4) S). This particular device in live-wood eating termite is perhaps meant for cutting the heartwood of the tea bushes. The maxillary palp shows the presence of a large number of sensilla, consisting of simple cuticular spines, sensilla trichodea and sensilla basiconica (Plate 4(5) SB, ST).

All the portions of the mouth parts, i.e. hypopharynx, maxillary palp, labial palp and labrum shows almost similar types of sensilla. Three different types of sensilla can be recognised (Plate 4(5) ST, SB, SC). These are sensilla trichodea, about 35–60 μm long 3–5 μm broad with well developed socket (about 7 μm in diameter), sensilla basiconica, about 10 μm long and 3 μm broad, and sensilla chaetica, about 34–50 μm long and 2–3 μm broad. The sensilla trichodea and sensilla basiconica have been observed to be more or less of the same width, but the sensilla trichodea is longer. Sensilla chaetica, on the other hand is more or less of the same length as that of sensilla trichodea, but its width is much less.

The antennae are twelve segmented. Excepting the terminal segment, all the segments are short and condensed. All the segments except the scape has been found to contain sensilla trichodea, sensilla chaetica and sensilla basiconica (Plate 4(2) ST, SC, SB). While sensilla trichodea and sensilla chaetica are localised in the lateral portion of each segment, the basiconic sensilla are present on the central portion. The sensilla trichodea are thicker (3 μm) than sensilla chaetica (1.8 μm), but both of them are comparatively longer (i.e. sensilla trichodea 27 μm in length and sensilla chaetica 48 μm in length) than sensilla basiconica (9 μm in length). The width of the sensilla basoconica is about 1.8 μm.

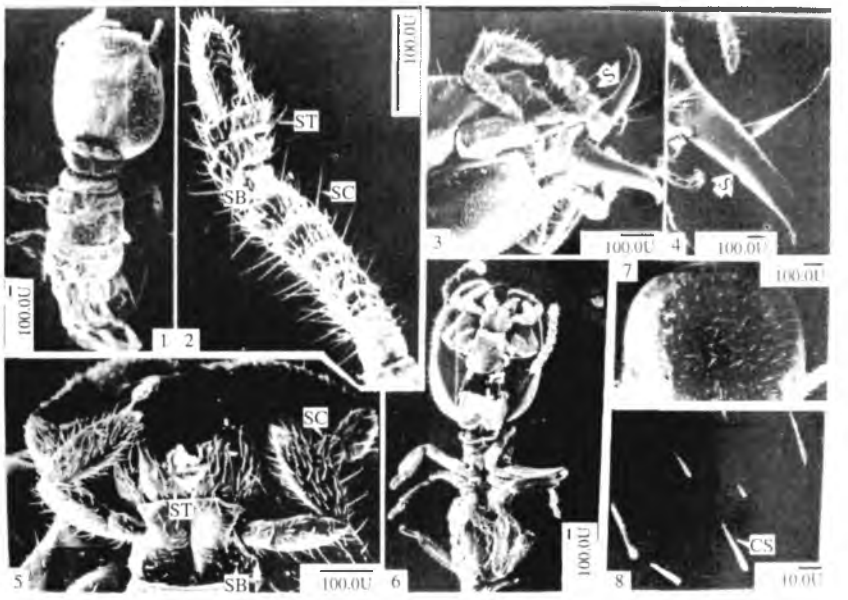

PLATE 4. Live-wood eating termite (*Microtermes obesi*, Holmgreen) ((1) to (5): Soldier, (6) to (8): Worker) (1) Entire body in dorsal view (2) Antenna showing sensilla trichodea (ST), Sensilla chaetica (SC) and Sensilla basiconica (SB) (3) Dorso-lateral view of mouth parts showing serrated inner edge of mandible (S) (4) The inner edge of mandible showing prominently the serrations (S) (5) Mouth parts in ventral view showing sensilla trichodea (ST), sensilla basiconica (SB) and sensilla chaetica (SC) (6) Entire body of worker in ventral view (7) to (8) Cuticular spines (CS) on dorsal surface of head.

Worker

General morphology of the body surface, appendages, antennae, mouth parts of the worker of live-wood eating termite (Plate 4(6)) is more or less same as that of soldier. The only significant difference has been observed in the head cuticles which are 3–25 μm long and the cuticular spines are sharp and pointed (Plate 4(7), (8) CS).

Scavenging termite (Odontotermes sp.)

Soldier

Scanning Electron Microscopy of the ventral surface of the mouth revealed all the parts distinctly (Plate 5(3)). The mandibles are well developed and are pointed at the tip. However, unlike the live-wood eating termite, the inner edge of the mandible is not serrated (Plate 5(3), (6) NS). The smooth, non-serrated inner edge in these termite is one of the examples of morphological adaptations, since, unlike live-wood eating termite, they are concerned with cutting dead/decomposed wood which are comparatively softer. Hypopharynx and labrum shows fewer sensilla as compared to those of live-wood eating termite. However, the maxillary palpi show the presence of a

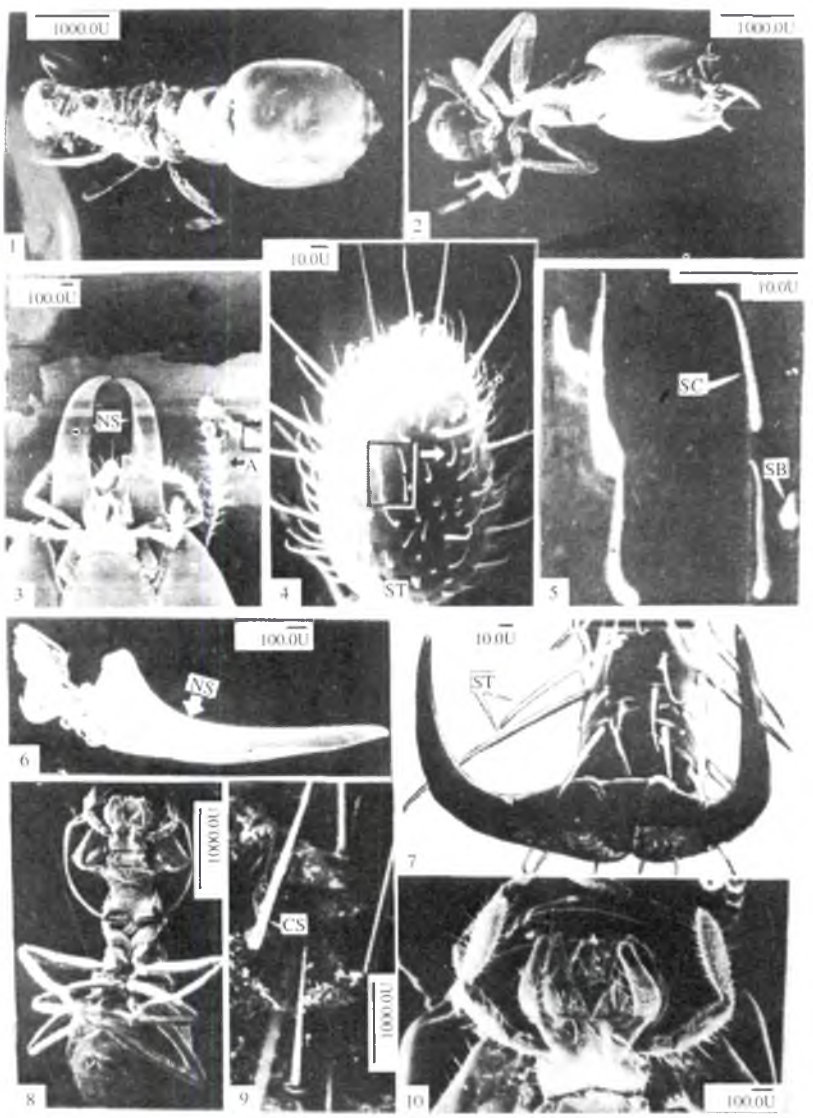

PLATE 5. Scavenging termite (*odontotermes* sp.) ((1) to (7): Soldier, (8) to (10): Worker) (1) and (2) Entire body in dorsal and ventral views respectively (3) Ventral view of mouth parts showing non serrated inner edge of mandible (NS) and antenna (A) (4) Tip of antenna showing different types of sensilla (5) Enlarged view of few sensilla chaetica (SC) and sensilla basiconica (SB) (6) The non-serrated inner edge (NS) is prominently visible in one mandible dissected out from the pest body (7) Tip of one leg showing several sensilla trichodea (ST) and the tip of tarsus shows one pair of claws (8) Entire body of a worker in ventral view (9) Dorsal surface of head showing cuticular spines (CS) (10) Mouth parts in ventral view showing different types of sensilla.

large number of sensilla trichodea and sensilla chaetica; but sensilla basiconica, could not be detected (Plate 5(3)).

The general organisation of antennal morphology is roughly similar to that of live-wood eating termite. However, the antennal segments are fifteen in number (Plate 5(3) A) and are longer and elliptical in comparison to those of live-wood eating termite which are shorter and condensed. The tip of the antenna showed the presence of a large number of sensilla trichodea, sensilla basiconica and sensilla chaetica. The length of the three types of sensilla has been found to be about 71, 3–4 and 28–30 μm respectively and the breadth is about 3, 2 and 2 μm respectively (Plate 5(4), (5) ST, SC, SB). The basiconic sensilla (SB) with swollen non-flexible base and blunt tip suggests their chemoreception and/or olfactory role (Plate 5(4), (5)).

The thoracic appendages of scavenging termite are more or less similar to those as observed in live-wood eating termite. However, the tarsal segments are characterised by the presence of only one type of sensilla, i.e. sensilla trichodea (Plate 5(7) ST). These sensilla show well developed socket, about 7 μm in diameter. The surface of the sensilla show striations, indicative of porous nature. The base of the sensilla are flexible. The tip of the tarsus shows the presence of a pair of well developed claws. Cuticular foldings and ventral companiform sensilla are present on the joint regions of the claw (Plate 5(7)).

The general body cuticle, particularly, the ventral region is marked by the presence of very few sensilla (Plate 5(2)). However, the dorsal region (Plate 5(1)) shows a few sensilla (mostly cuticular spines), although their numbers are much less than that of live-wood eating termite.

Worker

Gross morphology of the entire body, organisation of mouth parts and sensory system of the worker of scavenging termite has been found to be similar to those as described in the context with the live-wood eating termite. However, some differences exist in the finer ultrastructural details. Hypopharynx contain some long (about 200 μm) sensilla trichodea (Plate 5(10)). A few very long (1750–2400 μm) cuticular spines are also present on the dorsal surface of the head (Plate 5(9) CS). The labial palpi are shorter (330–340 μm) but thicker (100–160 μm) and contain a few sensilla basiconica (Plate 5(10)). Labium contain few sensilla trichodea (125 μm in length) with well developed sockets (12 μm in diameter) and a few sensilla basoconica (10–12 μm in length) (Plate 5(10)).

DISCUSSION

The ultrastructural findings on the various structures of tea pests conform the ecophysiological observations (i.e. the relationship among pest incidence/severity, infestation of the different plant parts, etc.) with humidity, temperature and soil conditions since structural modifications are important for interacting with the plant parts and perception of different types of stimuli (Dey, 1999).

In aphid, the labium is long and has attained the required modifications for sucking sap from the shoot. The bluntness of the tip suggest that piercing of the shoot is not needed for sucking sap, but in fact, laceration of the tender shoots by the tip is enough for the liberation of sap. The presence of sensilla trichodea and sensilla basiconica (Plate 1(4), (5) ST, SB) on the mouth parts suggest the presence of chemo and gustatoreceptors. Sensilla chaetica present on the thoracic appendages are likely to be contact chemoreceptors (Plate 1(3) SC). The compact ommatidia (Plate 1(2) CE) on the compound eye indicates a very good vision which helps in the general behaviour of the insect.

In thrips, flexibility of antennae and presence of sensilla basiconica suggests chemoreceptive and gustatory role performed by it in response to stimuli received from different directions of the substrate (Sliffer, 1970). The anchor like structure at the tip of the leg is also an important morphological adaptation which helps the insect in adhering itself to the plant parts. In fact, these structures cause laceration on the rolled young tea leaves, which persist as two to four permanent sand papery lines on the ventral surface of the mature leaves.

The sensory system in the red spider mite also shows significant correlation with their ecology and behaviour. The well developed sensilla chaetica and sensilla basiconica present in various parts of the body suggests that contact chemoreception and reception of volatile chemicals from the tea plants are carried out with sufficient precision. This is supported by their behavioural studies, where, it has been observed that, during heavy rain they are washed down to the lower zones of the bushes, but on subsequent withdrawal of rain, the pests again migrate to their original position (dorsal surface of the leaf) (Banerjee, 1965, 1972; Choudhury and Dutta, 1999). The presence of pits and some poreless sensilla with inflexible sockets may be related to hygro and thermoreception (Lacher, 1964; Altner *et al.*, 1983). The functional significance of these sensilla is confirmed by the characteristic predominance of the insect on dorsal surface of the tea leaves when the ambient temperature is high and therefore, the pest occur in large number/cause major damage during the summer months when the intensity of light and temperature is the maximum. The high light intensity, again, is associated with a problem for the mite species, since there is a likelihood of their visual prominence to the predators. It is highly interesting to note that some morphological adaptations of the general body cuticle might help the mite species to overcome this problem. The wavy striations of alternate electron dense and eletroluscent bands raised from the general cuticular plane, with more or less uniform periodicity and height may act as anti-reflection device, similar to corneal nipple present on the lens cuticle (Miller *et al.*, 1966; Dey, 1988, 1991; Dey and Dkhar, 1992).

Ecological and behavioural studies revealed that the live-wood eating termites devour the heart wood of living tea plants. Since the pest has a preference for the heart wood, it appears that they have some specific sensory mechanism to respond to the stimuli produced by phytochemicals from the tea plants. A large number of sensilla trichodea and sensilla chaetica with characteristic features of contact chemoreceptors suggests that these sensilla help the termite species to identify the

specific phytochemicals from the plant and also to differentiate between live and dead wood. The innumerable cuticular spines on the surface of head, thorax, abdomen and thoracic appendage are likely to perform protective role as and when the pest penetrates deep inside the tunnels within heart wood.

The serrated mandible of live-wood eating termite (soldier) appears to be an important adaptation for cutting the bark/wood of the living tea plant. The presence of a considerable large number of sensilla trichodea and sensilla basiconica on the maxillary palp suggests that chemoreception of volatile chemicals and contact chemoreception of various types of stimuli are used by the mouth parts before feeding activity starts. The shape, length and width of basoconic sensilla suggests their role in chemoreception and olfaction (Dey *et al.*, 1995). The sensilla trichodea on the other hand shows the characteristic features of chemoreception (Bland, 1983). The dimension, localised distribution and shape of the basiconic sensilla detected in the antenna has the characteristic features of sensory peg responsible for reception of moisture. These hygroreceptors were reported in many social insects, but there is no report on termite so far (Dietz and Humpheys, 1971). The presence of the hygroreceptors (Altner *et al.*, 1983) is related to the specific behaviour of the pest where the population abundance shows an inverse relationship with the soil moisture content.

An interesting correlation between morphology of mandible and feeding habit of termite species has been found. The presence/absence of serrated margins at the inner edge of mandible is directly related to the feeding behaviour of the pest. Since the scavenging termite does not basically devour the heart wood of living tea plants, therefore, unlike live-wood eating termite, it does not require serrated edge in the mandible. However, besides acting as a defensive organ, the pointed tip of the mandible is important for the pest to penetrate into the dead and rotten wood. The presence of fewer sensilla in the mouth parts of the scavenging termite suggests that it does not require the various stimulus from phytochemicals to be sensed. However, the presence of sensilla chaetica and sensilla trichodea suggests that contact chemoreception and mechanoreception may be well developed. The presence of sensilla basiconica, similar to hygroreceptor in the antenna, signifies the inverse relationship between the occurrence/severity of the termite sp. and the moisture content of the soil, similar to that observed in the live-wood eating termite. In thoracic appendages, the presence of only one kind of sensilla (i.e. sensilla trichodea) with well developed socket and surface striations suggest that the termite has a well developed contact chemoreceptor to detect the dead and rotten wood of the tea plant. The flexible base of the sensilla further supports this view (Sliffer, 1970). The presence of a pair of well developed claw on the tarsal tip, along with the occurrence of cuticular foldings and ventral campaniform sensilla on the joint region between claws and tarsus suggests that the claws are used for holding the substrates and can be moved at different angles (Schmidt and Smith, 1987).

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Isolation, Purification and Partial Characterization of Haemolymph Lipophorin from the Red Cotton Bug, *Dysdercus cingulatus* Fabr. (*Heteroptera: Pyrrhocoridae*)

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ABSTRACT: Lipophorin, a prominent insect plasma lipoprotein exists in several forms with respect to relative lipid content and apolipoprotein composition. Lipophorin was isolated from haemolymph of 2-day-old adult female, *Dysdercus cingulatus* by Density Gradient Ultracentrifugation. Purified lipophorin was subjected to dialysis and protein subunits were separated by Amicon cut off column. Purity of the lipophorin was confirmed by HPLC and SDS-PAGE. In HPLC, apolipophorin-I, apolipophorin-II and apolipophorin-III fractions were eluted as three peaks at about, 1.70, 2.08 and 3.05 minutes respectively. SDS-PAGE suggested that lipophorin is composed of three apoprotein subunits, apolipophorin-I (220 kDa), apolipophorin-II (80 kDa), apolipophorin-III (18 kDa).

KEYWORDS: Lipophorin, ultracentrifugation, dialysis, HPLC, SDS-PAGE.

INTRODUCTION

Studies on lipid transport in insects have made rapid progress during the past 40 years. The initial investigation in the locust by Tietz (1962) showed that acylglycerols are released from the fat body into the haemolymph. Chino and Gilbert (1964, 1965) first demonstrated that in the silkworm *Hyalophora ceceropia*, grass hopper *Melanoplus* and in the American Cockroach, *Periplaneta americana*, fatty acids are transported from the storage site, predominantly in the form of diacylglycerol associated with a specific haemolymph lipoprotein. Later these findings were confirmed by many investigators extending to a variety of species including locusts. Indeed, diacylglycerol serves to transport fatty acids from the site of storage and absorption to the site of utilization in almost all insect species (Downer and Steele, 1969; Gilbert and

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Chino, 1974; Chino and Downer, 1979; Langley *et al.*, 1981). Gilbert *et al.* (1965) called it 'high density lipoprotein' in view of its density $d = 1.15–1.17$ g/ml). Since then, several investigators have offered different other terms, like lipoprotein (Gilbert and Chino, 1974; Gellissen and Emmerich, 1980; Chino *et al.*, 1981a) diglyceride transporting lipoprotein, and diglyceride-binding lipoprotein (Gellissen and Emmerich, 1980).

In the year 1981b, Chino *et al.*, have developed a rapid and efficient method for purification of the diacylglycerol carrying lipoprotein from the haemolymph of the American Cockroach and locust. On the basis of these studies, it was concluded that insect diacylglycerol-carrying lipoprotein originally formed in the fat body serves to transport various polar and non polar lipids including diacylglycerols, cholesterol, hydrocarbons and carotinoids, from the sites of synthesis, to the site of utilization where these lipids are utilized as metabolic fuel. This lipoprotein serves multiple functions as a true carrier and acts as a reusable shuttle (Chino *et al.*, 1981a; Chino and Kitazawa, 1981; Sun *et al.*, 2000).

The research group involved in studying this lipoprotein have proposed a new term 'lipophorin' (from Greek lipos, fat: phoros, bearing) as a more appropriate generic term for this unique lipoprotein (Chino *et al.*, 1981a). In insects lipophorin (Lp) is the major haemolymph lipoproteins, which is composed of two apolipoproteins, apoLp-I = 230–250 kDa and apoLp-II = 70–85 kDa. A third small apo-protein (apoLp-III) of 18–20 kDa is associated with Lp in a reversible manner. In a high-density lipophorin (HDLp), one apoLp-I and one apoLp-II are associated with two apoLp-III molecules (Kawooya *et al.*, 1984, 1986; Ryan, 1990; Van der Horst, 1990; Surholt *et al.*, 1992; Blacklock and Ryan, 1994; Soulages and Wells, 1994).

MATERIALS AND METHODS

The red cotton bug *Dysdercus cingulatus* was reared in the laboratory at controlled condition. Eggs were removed every morning and kept in petri-dishes. When hatched, they were transferred to the rearing plastic basins and fed on soaked cotton seeds. Each morning newly emerged adults were isolated. These were considered as 0-day old. This method ensured availability of insects of known age group whenever required (Muraleedharan and Prabhu, 1978). Two-day old adult females were used for this study.

Collection of haemolymph

Haemolymph was collected by cutting the antennae and draining the haemolymph into an Eppendorf tube (graduated) with a sufficient quantity of phenylthiourea (PTU) as an anticoagulant. Haemolymph samples were diluted with Tris buffer (pH 6.8). This mixture was centrifuged at 5,000 rpm for 10 minutes at 4 °C (Charles *et al.*, 1992) in a refrigerated centrifuge (Universal 16 R Hettich, Zentrifugen Germany) to remove the haemocytes and other debris (Charles *et al.*, 1992). Supernatant was mixed with equal volume of sample buffer.

Protein determination

Protein content of haemolymph was estimated according to Lowry *et al.* (1951), using bovin serum albumin (BSA) as standard.

SDS-PAGE

The electrophoretic protein profile of haemolymph was determined by one dimensional sodium-dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE) carried out according to Laemmli (1970) under discontinuous and dissociating buffer system. The haemolymph protein samples were mixed with sample buffer (50 mM Tris-Hcl, pH 6.8, 2% (w/v) SDS, 10% (v/v) Glycerol, 0.1% bromophenol blue, 5% 2-mercaptoethanol) such that the protein concentration of each sample is 100 μg per well. The molecular weights of different bands in each sample were determined from the straight line, drawn between log molecular weight and R_m values.

Molecular weight determination

Molecular weight standards (HMWS, Genei) Myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa) and Egg albumin (45 kDa) were run in parallel lanes. A straight line, constructed from their relative migration and known molecular weights, was used to estimate the molecular mass of lipophorin subunits.

KBr density gradient ultra-centrifugation

For large scale separation of lipoprotein, method developed by Jose (2000) was used. Speed: 70000 rpm, Temperature: 7 °C, Time: 1 h and 30 min, Acceleration: 9 (slowest), Deceleration: 0 (no brake). The entire spin took 2 h. Preparative ultracentrifugation of lipoprotein has been carried out to isolate, purify and characterise lipoprotein particles. After centrifugation, the sample was fractioned into 150 μl of each from the top to the bottom and weighed. Refractive indices were determined with a refractometer. Refractive index was used to calculate the density. Absorbance at 450 nm of each fraction was determined. 20 μl of sample was taken from each fraction and subjected to SDS-PAGE with molecular markers. From SDS-PAGE, the band corresponding to 220, 80 and 18 kDa samples were pooled together from the aliquot. This sample was dialysed (Haunerland *et al.*, 1987) to remove KBr and used for subsequent SDS-PAGE and HPLC analysis.

Protein purification

Semipurified protein sample (after density gradient ultracentrifugation) was further purified using Amicon-cut-off protein concentrator (Ultrafree centrifugal filtration devices). Pore size of the membrane was 50 kDa, (50,000 NMWL, Ultrafree CL. 2.0 ml). Protein sample was taken in the upper tube and centrifuged at 3,500 rpm for 30 min at 4 °C. Sample present in the upper tube contains lipophorin I and II having molecular mass 220 and 80 kDa respectively. The sample present in the lower

tube contains lipophorin III having molecular weight of 18 kDa. Both samples were subjected to SDS-PAGE and HPLC fractionation for confirmation.

HPLC separation

The sample obtained by ultra-centrifugation was dialysed and adjusted to a protein concentration of 0.5 mg/ml. An aliquot containing 25 μg of protein was subjected to reverse-phase HPLC using a 4.6 × 250 mm waters C18 column (Waters, USA). The solvent system was 0.1% (v/v) trifluoracetic acid in water as aqueous phase and 0.085% Trifluroacetic acid in acetonitrle as organic phase. The flow rate was 1 ml/min. uv absorption was monitored at 280 nm. Apolipophorin (Sigma, USA) was used as standard. Purified haemolymph sample was diluted in 1 : 1 dilution with mobile phase and 100 μl of the sample was injected. The proteins eluted as three peaks at about 1.70, 2.08 and 3.05 min.

RESULTS

Density gradient ultra–centrifugation

Three fractions of lipophorin were isolated from the adult haemolymph. According to their hydrated densities, two of these lipophorins fall into the category of high density lipoproteins. They are called lipophorin-I (d. 1.05–I.150 g/ml) and lipophorin-II (d. 1.152–I.160 g/ml). Since the third lipoprotein fraction floated only at a density of 1.265 g/ml, this falls under the category of very high density lipophorins and is called here lipophorin-III. All the three lipophorins are light yellow in colour presumably due to the presence of carotenoids. Lipophorin appeared to be yellow in colour after KBr gradient ultra-centrifugation, due to the presence of carotenoids absorbed through food. This band was collected with a Pasteur pipette and subjected to dialysis to remove the salts and then subjected to HPLC and SDS-PAGE.

SDS-PAGE

Molecular mass of the lipophorin from haemolymph of *Dysdercus cingulatus* was determined by SDS-PAGE revealed three polypeptide bands, apolipophorin-I (220 kDa) apolipophorin-II (80 kDa) and apolipophorin-III (18 kDa) (Fig. 1). A comparison of molecular weight of apolipophorin-I, II and III of different known insects with ***Dysdercus cingulatus*** are shown in Table I.

TABLE 1. Comparison of molecular weight of apolipophorin I-II-III of different known insects with *D. cingulatus*

Sl. No.	Name of insect	Molecular weight	References
1.	Cochineal insect *Dactylopius confuses*	I-25 kDa II-22 kDa	Ziegler *et al.*, 1995
2.	Weevil	I-226 kDa	Shapiro, 1988

Sl. No.	Name of insect	Molecular weight	References
	Diaprepes abbreviatus	II-72 kDa	
3.	Tobacco horn worm *Manduca sexta*	I-245 kDa II-78 kDa	Shapiro *et al.*, 1984
4.	Fall web worm *Hyphantria cunea*	I-230 kDa II-80 kDa	Yun *et al.*, 1994
5.	House fly *Musca domestica*	I–IV 20 to 26 kDa	De and Capurro, 1991
		I-253 kDa II-85 kDa	Antonio *et al.*, 1987
6.	Locust	I-250 kDa	Weers *et al.*, 1993a Van der Horst *et al.*, 1992, 1993
	Locusta migratoria	II-72 kDa	Weers *et al.*, 1993b
7.	Silkworm *Bombyx mori*	I-270 kDa II-83 kDa I-250 kDa II-90 kDa	Miura and Shimizu, 1988 Kim and Kim, 1996 Toru *et al.*, 1995
8.	Mosquito *Aedes aegypti*	I-200 kDa II-66 kDa I-240 kDa II-75 kDa III-17 kDa I-204 kDa II-75 kDa	Dhadialla and Raikhel, 1990 Sun *et al.*, 2000 Ford and Van Heusden, 1994 Sun *et al.*, 2000
9.	*Hyalophora cecropia*	II-80 kDa III-17 kDa	William *et al.*, 1991
10.	Drosophila *Drosophila melanogaster*	I-240 kDa II-75 kDa I-275 kDa II-76 kDa	Pho *et al.*, 1996 Germain *et al.*, 1988
11.	*Manduca sexta*	I-285 kDa II-81 kDa	Nikhil *et al.*, 1978
12.	Fall Web worm *Hyphantria cunea*	I-230 kDa II-80 kDa III-18 kDa	Yun *et al.*, 1994 Yun and Kim, 1996
13.	Wax moth *Galleria mellonella*	I-240 kDa II-80 kDa	Wiesner *et al.*, 1997

Sl. No.	Name of insect	Molecular weight	References
		III-18 kDa	Imura *et al.*, 1998
		III-17.2 kDa	Halwani and Dunphy, 1999
		II-85 kDa	Donald *et al.*, 1994
14.	Common cut worm *Spodoptera litura*	I-235 kDa II-79 kDa	Jeon Byung *et al.*, 1998
15.	*Schistocerca gregaria*	I-224 kDa II-81 kDa III-20 kDa	Ogoyi *et al.*, 1995
16.	Honey bee *Apis mellifera*	I-250 kDa II-80 kDa	Robbs *et al.*, 1985
17.	Tsetse fly *Glossina morsitans*	I-250 kDa II-80 kDa	Ochanda *et al.*, 1991
18.	Giant waterbug *Lethocerus medius*	III-19 kDa	Kanost *et al.*, 1995
19.	Sheep Blowfly *Apis mellifera*	I-228 kDa II-80 kDa	Robbs *et al.*, 1985
20.	*Blattella germanica*	I-212 kDa II-80 kDa	Veeresh *et al.*, 1999
21.	*Triatoma infestans*	II-77 kDa	Maria *et al.*, 1991
22.	Southwestern corn borer *Diatraca grandiosella*	I-220 kDa II-74 kDa III-17 kDa	Charles *et al.*, 1992
		I-220 kDa II-82 kDa	Venkatesh *et al.*, 1987
23.	*Agrius convolvui*	III-20 kDa	Yun, 2000
24.	*Gastrimargus africanus*	I-250 kDa II-80 kDa III-20 kDa	Haunerland *et al.*, 1987
25.	*Acherontia atropos*	I-240 kDa II-70 kDa III-20 kDa	Surholt *et al.*, 1988
26.	**Red cotton bug *Dysdercus cingulatus***	**I-220 kDa II-80 kDa II-18 kDa**	**Present study**

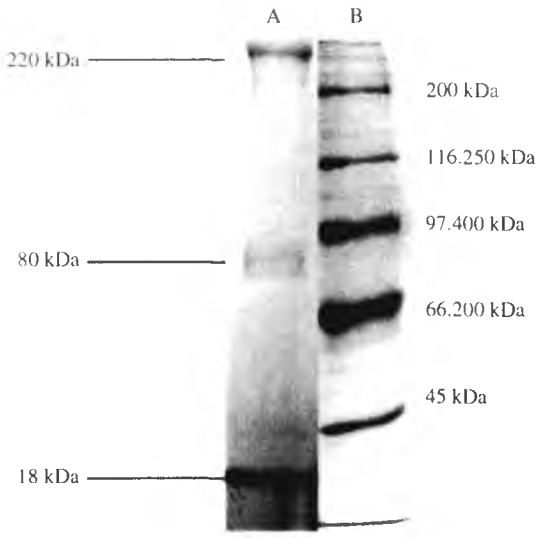

FIGURE 1. Electrophoregram showing the purified Haemolymph Lipophorin fraction

Determination of molecular mass

The apparent molecular weight of the monomer of purified lipoprotein was determined by comparing its rate of migration on SDS-PAGE with the rates of known protein standards and making reciprocal plot (Lambin *et al.*, 1976), Fig. 2.

Density of Lipophorin

The density of lipophorin purified from *Dysdercus cingulatus* plasma on a KBr density gradient was estimated as apoLp-I (1.07 g/ml), apoLp-II (1.15 g/ml) and apoLp-III (1.26 g/ml) which is in close agreement with the published values of 1.105 g/ml (Kurian, 1978); 1.12 g/ml (Ryan *et al.*, 1984), 1.10 g/ml (de Kort and Koopmanschap, 1987), 1.16 g/ml (Germain *et al.*, 1988), 1.08 g/ml (Shapiro, 1988), 1.11 g/ml (Sheppy and Stanley, 1992), 1.108 g/ml (Gu *et al.*, 1995), 1.19 g/ml (Srivastava and Thangavelu, 1996), 1.109 g/ml (Veeresh *et al.*, 1999), 1.112–1.114 g/ml (Sun *et al.*, 2000). Lipophorin density of *D. cingulatus* with other insects were shown in Table 2.

HPLC

Isolation of apolipophorin from haemolymph using HPLC was as previously described. (Van der Horst *et al.*, 1991; Charles *et al.*, 1992; Slovak and Repka, 1993; Donald *et al.*, 1994; Ziegler *et al.*, 1995; Wiesner *et al.*, 1997; Christoph *et al.*, 1998). In reverse phase HPLC using C18 column, lipophorin eluted as three sharp peaks, at 1.70, 2.08 and 3.06 min. respectively (Fig. 3).

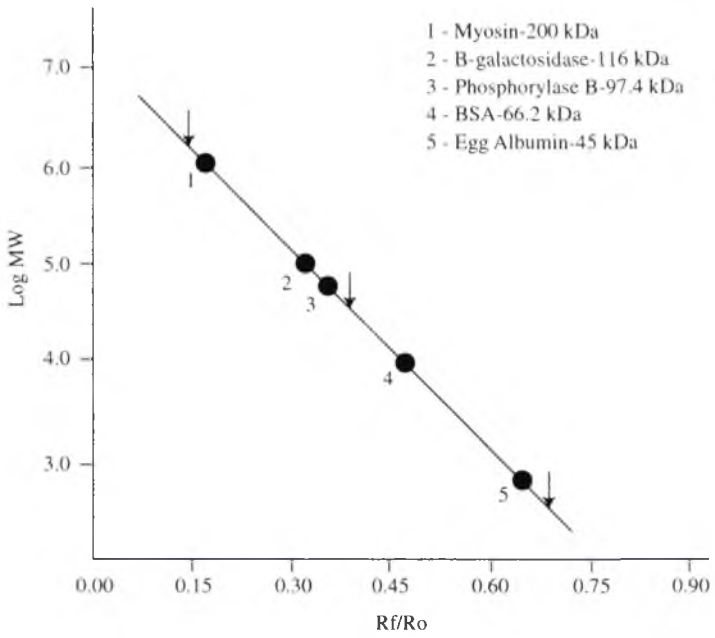

FIGURE 2. Reciprocal plots of migration of Lipophorin subunits during SDS-PAGE relative to known standards. Arrows indicate the molecular weight of apoLp-I-II & III.

DISCUSSION

In insects as in other organisms, lipids have got many fundamental functions including structural, functional, behavioural and energetic roles. Their transport in an aqueous environment is carried out by lipophorin, ubiquitous and major lipoprotein in insect haemolymph. Lipophorin is a dynamic particle that acts as a reusable shuttle, loading lipids, at the sites of absorption and unloading them at storage organs or peripheral tissues. Lipophorin performs these functions without degradation of its basic matrix structure, cycling through lipid-rich and lipid-depleted states (Beenakkers *et al.*, 1988; Van der Horst *et al.*, 1993).

Lipophorin, which usually constitutes the single lipoproteins in insect haemolymph may exist in several forms with respect to relative lipid content and apolipophorin composition (Beenakkers *et al.*, 1988). Generally lipophorin consist of apoLp-I, -II and -III, the molecular weight of which are 220–250 kDa, (Fernando-Warnakulasuriya *et al.*, 1988), 75–80 kDa (Chino, 1985; Kanost *et al.*, 1990; Soulages and Wells, 1994) and 18–20 kDa (Surholt *et al.*, 1992) respectively (Chino and Kitazawa, 1981; Shapiro *et al.*, 1984; Ryan *et al.*, 1984; Kawooya *et al.*, 1984; Surholt *et al.*, 1992). Lipophorin of *D. cingulatus* isolated in the present study comprised of apoLp-I, -II and -III with molecular weight estimated to be 220, 80 and 18 kDa respectively. This result confirmed earlier observations (Kurian, 1978; Shapiro *et al.*, 1984; Van der Horst *et*

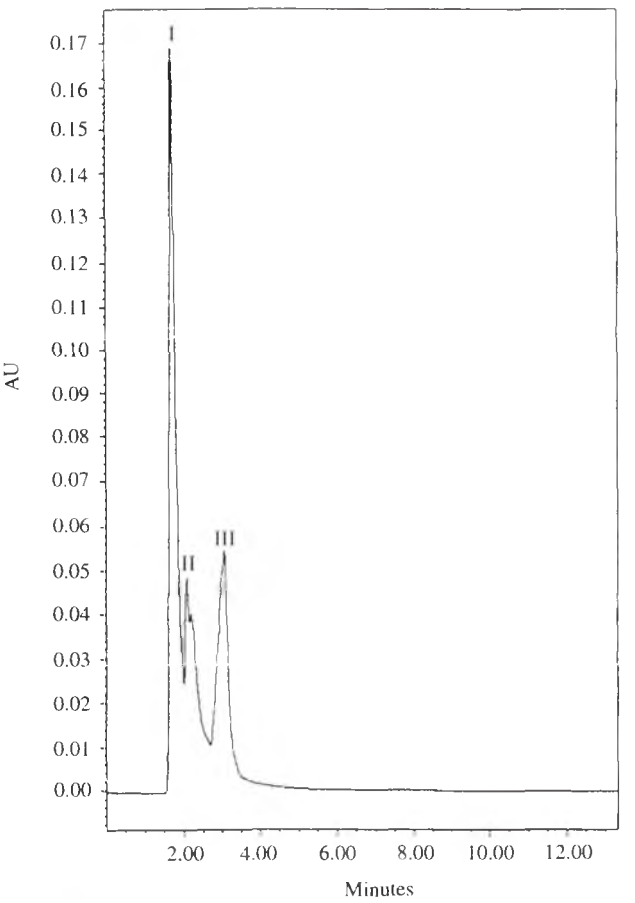

FIGURE 3. HPLC profile showing three different peaks of apolipophorin fractions. ApoLp-I Retention time 1.73; ApoLp-II Retention time 2.184; ApoLp-III Retention time 3.059.

al., 1991; Charles *et al.*, 1992; Wiesner *et al.*, 1997) that the molecular weight of sub units were as apoLp-I m 220 kDa, apoLp-II m 80 kDa and apoLp-III. m 18 kDa. In reverse-phase HPLC lipophorin eluted as three sharp peaks, apoLp-I, -II and -III at about 1.70, 2.08 and 3.05 mins respectively.

Insect lipophorin characterized to have a density of 1.063–1.210 g/ml (Ryan *et al.*, 1984; Beenakkers *et al.*, 1988). The density of *D. cingulatus* haemolymph lipophorin fraction falls within this range. The density of haemolymph lipophorin of *D. cingulatus* is apoLp-I = 1.07, -II = 1.15 and -III = 1.26 g/ml. This is in close agreement with the published values, (Kurian, 1978; Ryan *et al.*, 1986; Shapiro, 1988; de Kort and Koopmanschap, 1987; Gu *et al.*, 1995; Srivastava and Thangavelu, 1996; Veeresh *et al.*, 1999; Sun *et al.*, 2000).

TABLE 2. Comparison of density of haemolymph lipophorin of different known insects with *D. cingulatus*

Species	Density (g/ml)	References
Manduca sexta	1.10 g/ml	Engler *et al.*, 1997
Apis mellifera	1.11 g/ml	Sheppy and Stanley, 1992
Leptoglossus zonatus	1.09 g/ml	Veeresh *et al.*, 1999
Periplaneta americana	1.12 g/ml	Ryan *et al.*, 1984
Acheta domesticus	1.10 g/ml	de Kort and Koopmanschap, 1987
Tenebrio molitor	1.12 g/ml	Ryan *et al.*, 1984
Drosophila melanogaster	1.12 g/ml	Pho *et al.*, 1996
Gastrimargus africanus	1.10 g/ml	Haunerland *et al.*, 1987
Hyalophora cecropia	1.24 g/ml	Telfer *et al.*, 1991
Acherontia atropos	1.13 g/ml	Surholt *et al.*, 1988
Diatraea grandiosella	1.11 g/ml	Venkatesh *et al.*, 1987
Papilio polyxenes	1.13 g/ml	Ryan *et al.*, 1986
Aedes aegypti	1.10 g/ml	Van Huesden *et al.*, 1997
Rhodnius prolixus	1.11 g/ml	Coelho *et al.*, 1997
Locusta migratoria	1.12 g/ml	Weers *et al.*, 1992
Podisus maculiventris	1.16 g/ml	Haunerland *et al.*, 1994
Musca domestica	1.10 g/ml	Antonio *et al.*, 1987
Dysdercus cingulatus	**ApoLp-I = 1.07 g/ml** **ApoLp-II = 1.15 g/ml** **ApoLp-III = 1.26 g/ml**	**Present study**

Germain *et al.* (1988) demonstrated that lipophorin is composed of 62.5% protein, 23.1% phospholipid, 7.4% diacylglycerol, 5.4% triacylglycerol, 0.9% hydrocarbon and 0.7% sterol.

It thus seems that the haemolymph lipophorins in *D. cingulatus* generally resemble the lipophorins of other insect species in most of their physical and biochemical characteristics such as ultracentrifugal behaviour, electrophoretic properties and HPLC fractions. It may be that they are similar in function as well which is to be established in subsequent studies.

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Influence of Environmental Factors on Indoor Resting *Culex quinquefasciatus* in Tea Agro-ecosystem of Assam, India

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ABSTRACT: A total of 8,892 *Culex quinquefasciatus* mosquitoes were collected spending 288 man hours in the study gardens in two consecutive years. Highest density of indoor resting *Cx. quinquefasciatus* was recorded in the month of March 1996 and lowest in the month of January 1997. Overall parity rate was 48.4%. Temperature had a profound negative influence on parity status of these mosquitoes. Resting density of mosquitoes had curvilinear relationship with the rainfall. Environmental temperature was found to have significant influence over indoor resting density of the vector, whereas relative humidity did not seem to influence it in the study tea gardens. The minimum temperature was best among the subset of variables for selecting most suitable model to explain the resting density variations of this vector in tea agro-ecosystem ($R^2 = 0.599$; $F = 14.9399$; $P = 0.0001$). Previous months rainfall and minimum temperature of current month was found to be the next suitable model to explain the variation of vector density ($R^2 = 0.794$; $F = 0.6309$; $P = 0.0002$). The abundance of indoor resting *Cx. quinquefasciatus* was temporally bimodal in the study gardens. Besides minimum and maximum temperature, rainfall and the complex interaction between all these environmental factors greatly influence the mosquito density.

KEYWORDS: climatic influence, filaria vector, Assam.

INTRODUCTION

The prevalence of bancroftian filariasis among the tea garden workers of Assam, has been reported to be high (6–11% microfilaraemia and 1.8–3.8% chronic filariasis). Except a few scattered reports of spot surveys (Rao, 1942; Basu, 1957; Raina *et al.*, 1993; Dutta *et al.*, 1995) of filariasis and its vectors, no systematic longitudinal study

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on vector bionomics has ever been conducted in tea agro ecosystem of Assam. The region is characterized by absence of dry hot summer and receives high and wide spread rainfall due to south west monsoon throughout the year. Relative humidity remains high throughout the year. Morning humidity was comparatively more than the evening relative humidity during the study period.

In tea gardens all the families of permanent employees live in colonies, popularly known as labour lines and form a part of the ecology. Each labour line is normally a congregation of several quarters. Tea plantations are perennial agro-crop. In tea garden areas increased use of insecticides and pesticides to protect plantation, vector population is expected to be influenced greatly. Local epidemiology of filariasis is strongly influenced by the behaviour and ecology of various species of vector mosquitoes. Changes in the environmental conditions of different places affect the population densities and survival rates of vector mosquitoes. Climatic conditions influence the infection and development of the parasite in *Cx. quinquefasciatus* (Kaul and Wattal, 1968; Rajagopalan, 1980). Further vector density acts as the compensating factor in low infectivity area. Hence studying vector prevalence and its temporal variation in this unique agro-ecosystem is expected to give an insight of filaria transmission in the garden workers.

MATERIALS AND METHODS

Various methods followed for studying the vector density were essentially as described by World Health Organisation (1975) and National Filaria Control Programme Operational Manual (1978). For longitudinal study two tea gardens were selected randomly and on operational feasibility from upper Assam. Adult mosquitoes were collected from 12 collection sites (6 fixed and 6 randomly selected each time) in every week from September 1995 to August 1997.

Battery operated aspirators or suctions tubes operated by mouth, were used to collect the mosquitoes. Collected mosquitoes were transported to the main laboratory; identified and counted species wise and their abdominal conditions were noted. MHD of female and male *Cx. quinquefasciatus* were also worked out separately. Representative samples of mosquitoes were dissected for parity status (Detinova, 1962). Information on monthly average of maximum and minimum temperature, morning and evening relative humidity, total amount of monthly rainfall and total number of rainy days in a month was collected from Toklai Tea Research Centre at Dikom of Dibrugarh District from September 1995 to August 1997. The influence of meteorological factors on the temporal variations in the abundance of morning resting density of *Cx. quinquefasciatus* was studied using linear, quadratic and cubic regression models with and without time lags.

RESULTS

A total of 8892 *Cx. quinquefasciatus* mosquitoes were collected spending 288 man-hours in the study tea gardens. Of these, 2452 were males and 6440 were females.

TABLE 1. Indoor reseting *Cx. quinquefasciatus* in tea agro-ecosystem of Assam and the meteorological parameters

Months	T_{max} °C	T_{min} °C	RH_{morn} %	Rainfall cms	R_{days} No.	Total Females (MHD)	Total Males (M:F)
Sep-95	30.2	23.7	95	57.36	22	256(21.3)	88(1:1.2)
Oct-95	31.2	20	94	5.67	11	448(37.3)	136(1:3.7)
Nov-95	28.6	16.4	95	3.87	4	391(32.6)	124(1:3.2)
Dec-95	24.3	12.1	96	1.26	4	144(12.0)	17(1:8.5)
Jan-96	22.3	10.5	97	3.12	8	152(12.7)	110(1:1.4)
Feb-96	26.1	12.1	94	5.24	8	208(17.3)	68(1:3.1)
Mar-96	26	16.4	91	21.58	18	465(38.75)	156(1:3.0)
Apr-96	29.1	18.9	86	20.7	18	300(25.0)	131(1:2.3)
May-96	28.2	21.3	92	62.23	24	238(19.8)	70(1:3.4)
Jun-96	32.1	24	87	26.39	19	251(20.9)	216(1:1.2)
Jul-96	30.3	24.3	95	51.18	27	264(22.0)	196(1:1.3)
Aug-96	32.2	25	94	38.89	24	235(19.6)	118(1:2)
Sep-96	32.2	24.4	93	21.99	17	229(19.1)	113(1:2)
Oct-96	30.7	20.4	94	17.44	11	396(33.0)	219(1:1.8)
Nov-96	28.5	15.8	94	0.24	1	308(25.7)	192(1:1.6)
Dec-96	26.9	9.3	95	0	0	221(18.4)	38(1:5.8)
Jan-97	23.5	8.6	95	3.08	6	121(10.1)	15(1:8)
Feb-97	21.4	11.3	94	6.56	12	229(19.1)	89(1:2.6)
Mar-97	25.5	15.3	92	17.21	18	344(28.7)	60(1:5.7)
Apr-97	27.9	17.4	88	9.7	12	305(25.4)	64(1:4.8)
May-97	30	21.2	91	19.38	17	208(17.3)	44(1:4.7)
Jun-97	30.8	23.1	94	54.36	22	272(22.7)	68(1:4)
Jul-97	33.6	24.6	93	49.09	24	223(18.8)	34(1:6.6)
Aug-97	34.3	24.7	91	32.23	14	232(19.3)	86(1:2.7)

T_{max} = Average Maximum Temperature; T_{min} = Average Minimum Temperature; RH_{morn} = Morning Relative Humidity; R_{days} = Rainy Days

The average monthly Man-Hour Density (MHD) during the study period varied from 10.1 to 38.75 (mean = 22.4; 95% CI = 17.7–27.0) for females and 11.33 to 51.75 MHD for both sexes together. Highest density of indoor resting *Cx. quinquefasciatus* was recorded in the month of March 1996 and lowest in the month of January 1997 (Table 1).

Based on the pooled data of two years the abundance showed prominent seasonal fluctuations of indoor resting female mosquitoes. The peak abundance of female mosquitoes was observed in the months of March (MHD = 33.7) and October (MHD = 35.2). On the other hand indoor resting male mosquitoes were less abundant. The MHD of adult males ranged from 2.3 to 14.8 (mean = 8.5; 95% CI = 6.2–10.9). The sex ratio was in favour of females ranged from 1.8 to 6.6 (mean = 3.0; 95% CI = 2.1–3.8).

A total of 6 regression models were used to test the relationship between indoors resting density and rainfall. The temporal variations between the rainfall and morning resting density of the vector showed a curvilinear relationship. This was best described

by quadratic regression model ($R^2 = 0.379$; $P = 0.0085$). The rainfall of both current and previous month had substantial effect on indoors resting density (Table 2). However, average rainfall of current and previous month above 274 mm had a limiting effect on their abundance.

Temperature also had significant effect on the abundance of indoor resting *Cx. quinquefasciatus*. The relationship between temperature and resting density was best described by second-degree polynomial regression equation. The fluctuation of mosquito abundance varied synchronously with minimum temperature except when the minimum temperature exceeds 20 °C. This curvilinear relationship is best visualized by the scatter diagram and the quadratic regression model was found to be most suitable one ($R^2 = 0.59$; $P = 0.0001$). Maximum temperature had lesser but statistically significant influence on resting density of *Cx. quinquefasciatus*. The relationship between the two was not linear and both quadratic ($R^2 = 0.28$; $P = 0.0335$) and cubic ($R^2 = 0.28$; $P = 0.0309$) regression models were found to be significant (Table 3). Relative humidity did not seem to influence the indoor resting vector density in the study tea gardens.

In order to study the influence of the above mentioned meteorological parameters collectively on indoor resting *Cx. quinquefasciatus* multiple regression models were used. The results are summarized in the Table 4. Using backward elimination strategy for selecting the best subset of variables it was found that the model containing only minimum temperature was the best model explaining the variations in densities of morning indoor resting *Cx. quinquefasciatus* ($R^2 = 0.599$; $F = 14.9399$; $P = 0.0001$). Second factor, which was considered, important was interaction between previous month's rainfall and minimum temperature of current month and is included in model two. This was found to be the next suitable model to explain the variation of vector density in the study garden ($R^2 = 0.794$; $F = 10.828$; $P = 0.0002$). In the third model in addition to parameters included in second model, maximum temperature, average rainfall {(rainfall of current month + rainfall of previous month)/2} and interaction between minimum temperature and current months rainfall were also included ($R^2 = 0.677$; $F = 5.588$; $P = 0.0027$).

Overall 48.4% of 1985 females of *Cx. quinquefasciatus* examined were parous. Parous rates ranged from 37.2 to 66.7% in different months. Out of these 30.3% were uniparous, 14.11% bi-parous, 3.58% triparous and 0.35% quadriparous. Parity rate was comparatively more in cooler months (December to February). Quadriparous mosquitoes were noted only during November to February. Temperature had a profound negative influence on parity status of these mosquitoes. About 51.5% of temporal variation in parity status were explained by minimum temperature ($R = -0.72$; $F = 20.84$; $P = 0.0002$). Similarly maximum temperature also exerted negative influence on parity status ($R = -0.7$; $F = 21.24$; $P = 0.0002$). The relation between rainfall and parity were also negatively correlated. The second-degree polynomial regression explained 37.9% of variation in parity ($R^2 = 0.379$; $P = 0.085$).

TABLE 2. Regression models describing relationship between indoor morning resting density of *Cx. quinquefasciatus* and rainfall (in cms). Dependent variable is transformed into log (MHD + 1)[a]

Regression model no.	Independent variables	β	S.E. of β	β coefficient	R	R^2	F-stat	P-value
1	Rain	4.78E-04	0.001455	0.0699	0.07	0.005	0.108	NS
	Constant	1.3373	0.043019					
2	Rain square	1.21E-04	8.80E-05	−1.06259	0.295	0.087	0.999	NS
	Rain	0.00748	0.00529	1.09302				
	Constant	1.2888	0.05499					
3	Rain $(t-1)^b$	0.001486	0.001466	0.21595	0.216	0.047	1.0272	NS
	Constant	1.31574	0.0433171					
4	Rain $(t-1)$ square	−1.30E-04	4.15E-05	−1.143	0.366	0.134	1.55	NS
	Rain $(t-1)$	0.009083	0.005528	1.32				
	Constant	1.26459	0.0554					
5	(Rain $(t-1)$ + Rain)/2[c]	0.00143	0.00178	0.172418	0.172	0.029	0.643	NS
	Constant	1.317721	0.047805					
6	(Rain $(t-1)$ + Rain/2)^2	−4.13E-04	1.23E-04	−2.451571	0.616	0.379	6.1149	$P = 0.0085$
	(Rain $(t-1)$ + Rain)/2	0.02116	0.006058	2.551592				
	Constant	1.14766						

Dependent variable is transformed to Log(MHD + 1)[a] to stabilize variance; Sampling period is 24 months (Sep'95 to Aug'97); [a]MHD = Main Hour Density; [b]Rainfall lagged by one month; [c]Average rainfall of current and previous month; $Significance of regression model as whole based on ANOVA; R^2 = Proportion of variation in indoor resting density (MHD) explained by the model; R = Correlation.

TABLE 3. Regression models describing relationship between indoor resting density of *Cx. quinquefasciatus* and temperature

Regression model no.	Independent variables	β	S.E. of β	β coefficient	R	R^2	F-value	P-value
1	T_{max}	0.012746	0.007953	0.323339	0.323	0.105	2.568	NS
	Constant	0.983621	0.22888					
2	T_{max}	0.2584	0.110259	6.556624	0.526	0.276	4.011	$P = 0.0335$
	T^2_{max}	−0.004406	0.001973	−6.247057				
	Constant	−2.388356	1.524366					
3	T_{max}	0.138591	0.05574	3.51572	0.531	0.282	4.122	$P = 0.0309$
	T^3_{max}	−5.34E-05	2.34E-05	−3.220036				
	Constant	−1.3146	1.030794					
4	T_{min}	0.007884	0.005056	0.315477	0.315	0.0995	2.43156	NS
	Constant	1.202866	0.096894					
5	T_{min}	0.150634	0.028062	6.027	0.7746	0.5999	15.749	$P = 0.0001$
	T^2_{min}	−0.004097	0.000799	−5.7554				
	Constant	0.082701	0.228315					

Dependent variables transformed to log(MHD + 1) to stabilize variance; T_{max} = Maximum temperature; T_{min} = Minimum temperature; Sampling period of 24 months from Sep'95 to Aug'97.

TABLE 4. Multiple regression models describing relationship between indoor resting density of *Cx. quinquefasciatus* and climatic parameters (Dependent variable transformed to log(MHD + 1)

Regression model no.	Independent variables	β	S.E. of β	β coefficient	R	R^2	F-value	P-value
1	Average rain^2	−3.23E-04	2.48E-04	−1.9159	0.823	0.677	5.588	0.0027
	T_{max}	7.73E-06	7.29E-06	0.4646				
	T_{min}	0.07262	0.019102	2.8438				
	T_{min} cube	−1.10E-04	2.67E-05	−4.2478				
	Inter 1	4.81E-04	3.71E-04	1.5798				
	Inter 3	5.18E-04	3.54E-04	1.8474				
	Constant	0.441258	0.193706					
2	T_{min}	0.87928	0.15725	3.4433	**0.794**	0.634	10.828	0.0002
	T^3_{min}	−9.14E-05	1.83E-05	−3.515				
	Inter 3	1.02E-04	7.98E-05	0.36447				
	Constant	0.390373	0.16989					
3	T_{min}	0.084028	0.015674	3.29059	**0.774**	0.599	14.94	0.0001
	T_{min} cube	−7.93E-05	1.60E-05	−3.0507				
	Constant	0.419329	0.171067					

Averagerain 2 = Square of (Rainfall of the month + rainfall of previous month)/2; T^3_{max} = Cube of average maximum temperature in each month; T_{min} = Average minimum temperature of the month; T^3_{min} = Cube of the average minimum temperature of each month; Inter 1 = Minimum temperature × Rainfall (in cms) of the month; Inter 3 = Minimum temperature × Rainfall of the previous month

DISCUSSION

The abundance of indoor resting *Cx. quinquefasciatus* was temporally bimodal in the study gardens. The first peak was observed during March–April (pre-monsoon) and the second during October–November (post-monsoon). The pre-monsoon peak was found to be more prominent. Peak period of abundance of indoor resting *Cx. quinquefasciatus* has been reported to vary from place to place. This may be explained on the basis of differences in climatic conditions of the places (temperature, humidity and rainfall). In Ernakulam (Kerala) peak abundance is between January to March (Pal *et al.*, 1960), in Mangalore during June to September (Subramanian and Thampi, 1958), in Rajamundry (Andhra Pradesh) during November to January (Dhar *et al.*, 1968) and in Barabanky (Uttar Pradesh) during March to April (Nanda *et al.*, 1962). In all these areas rainfall and temperature were reported to influence the density. De and Chandra (1994) reported that the density of indoor resting *Cx. quinquefasciatus* in Midnapur, West Bengal varied from 12 to 61.75 per man-hour. The highest density was found in the month of April but gradually started decreasing with the onset of rainy season. They found that with the onset of irregular showers during March–April there is increase in breeding places. They have related it with the popular notion of 'Kalbaishakhi' where irregular showers are accompanied by thunderstorm and high-speed wind. A similar phenomenon, 'Bordoishilla' is also noted in the present study area, which is equivalent to 'Kalbaishakhi' of Bengal. This period also gives rise to multiplicity of breeding places, which helps in building up of mosquito density (March, April). Dibrugarh is a high rainfall area with long spells of rainy days. In the study the relationship between the rainfall and morning resting density of the vector was found to be curvilinear and was best described by quadratic regression model. The garden environment become receptive for vector breeding after the rain starts from February–March onward. Creation of new breeding sites along with the existing ones (wastewater collection) make the impact perceptible on vector density and it requires about a month in these gardens. During pre-monsoon season or just before onset long spells of rainy days, irregular showers help in formation of small collections of water which facilitate mosquito breeding. The density of *Cx. quinquefasciatus* significantly decreases in winter possibly due to drying up of breeding habitats as a result of less rainfall and also for the limiting effect of minimum temperature. In tropical climate temporal variation of mosquito populations is closely related to available moisture either in the form of annual precipitation or irrigation (Reisen *et al.*, 1976). The effect of rainfall on mosquito population varies accordingly to its amount and the physical condition of the terrain. Exceptionally heavy rainy season might be favourable to the development of a number of species, yet detrimental to others. Repeated rains cause severe flooding, resulting in temporary flushing out of the breeding places. Consequently the breeding of vector population is greatly reduced but it will soon be reestablished when the normal conditions are restored (WHO, 1975). Minimum temperature explained about 60% variation in indoors resting mosquitoes. Minimum temperature varied between 8.6 to 24.7°C. Minimum temperature below 15°C acts as a limiting factor for mosquito development (Das, 1976). In the study

tea gardens minimum temperature goes below 15°C in winter, as a result resting *Cx. quinquefasciatus* densities might have gone low during this period. The mechanism of limiting effect of minimum temperature may be related to the lengthening of gonotropic cycle (Rajagopalan, 1980). The average maximum temperature was by and large conducive for mosquito proliferation throughout the year. The deterrence of maximum temperature beyond 33°C (Das, 1976) was found only occasionally in July and August. Hence the influence of maximum temperature in the abundance of *Cx. quinquefasciatus* was not very prominent. The male population among the indoor resting population of *Cx. quinquefasciatus* in tea gardens was found to be similar as reported elsewhere (Kanhekar, 1994; Dhar *et al.*, 1968).

In the present study, increases of parity in December and January are coincident with decrease in temperature. However, seasonal fluctuation and some increase in parity rate during June and July suggest that there is more than one factor in determining the parity status of indoor resting mosquitoes. Presence of older mosquitoes (4-parous) in winter months may be related to longer survival of *Cx. quinquefasciatus* during winter in the tea gardens. Increase in longevity of mosquitoes at low temperature has also been reported earlier (Pal, 1943).

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Taxonomy of Some Aphid Species (Homoptera: Aphididae) of Garhwal Range of Western Himalaya

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ABSTRACT: Two new species of aphids *viz.*, *Cryptomyzus elshotze* and *Vesiculaphis polygonii* infesting *Elshotzia* sp. and *Polygonum alatum*, respectively are described. Besides, a note on *Schoutedenia emblica andhraka* Hille Ris Lambers is provided from Garhwal range of Western Himalaya.

KEYWORDS: aphids, new species, subspecies, taxonomic note, Garhwal Himalaya, India.

TAXONOMIC ACCOUNT

***Cryptomyzus elshotze* sp. nov. (Figs 1 and 4) Apterous viviparous female**

Body 2.76–2.78 long and 1.29–1.37 mm wide. Head brown, both dorsum and venter with spinules; frons with moderately developed median tubercle; lateral frontal tubercles well developed and diverging; dorsum with 6 pairs of hairs; dorsal cephalic hairs thick with spatulate to capitate apices arising from tuberculate bases, longest one on vertex 0.060–0.068 mm long and 1.60–1.80 times the b.d.III. Antennae 6-segmented, 1.20–1.23 times the body length; segment I and II concolorous with head and faintly imbricated; rest of flagellum pale with little darker joints and gradually but distinctly imbricated apicad, segment I with 5–6 hairs, II with 3–4 hairs; longest one on segment III 0.022–0.026 mm long and 0.75–0.87 times the b.d.III; p.t. 4.20–4.35 times the bese of segment VI and 1.01–1.03 times the antennal segment III; segment III with 19–20 protuberant secondary rhinaria distributed over entire length of the segment. Rostrum extended to mid-coxae; ultimate rosrtral segment 1.50–1.55 times the h.t.2 bearing 36 secondary hairs. Abdomen dorsally little scabrous, dorsum with moderately long hairs with knobbed apices; anterior tergites with 10–12 hairs, longest on these tergites 0.033–0.040 mm long and 1.00–1.20 times the b.d.III; marginal hairs usually in a group of 3; tergites VII and VIII with 6–8 and 4–5 hairs; longest on these

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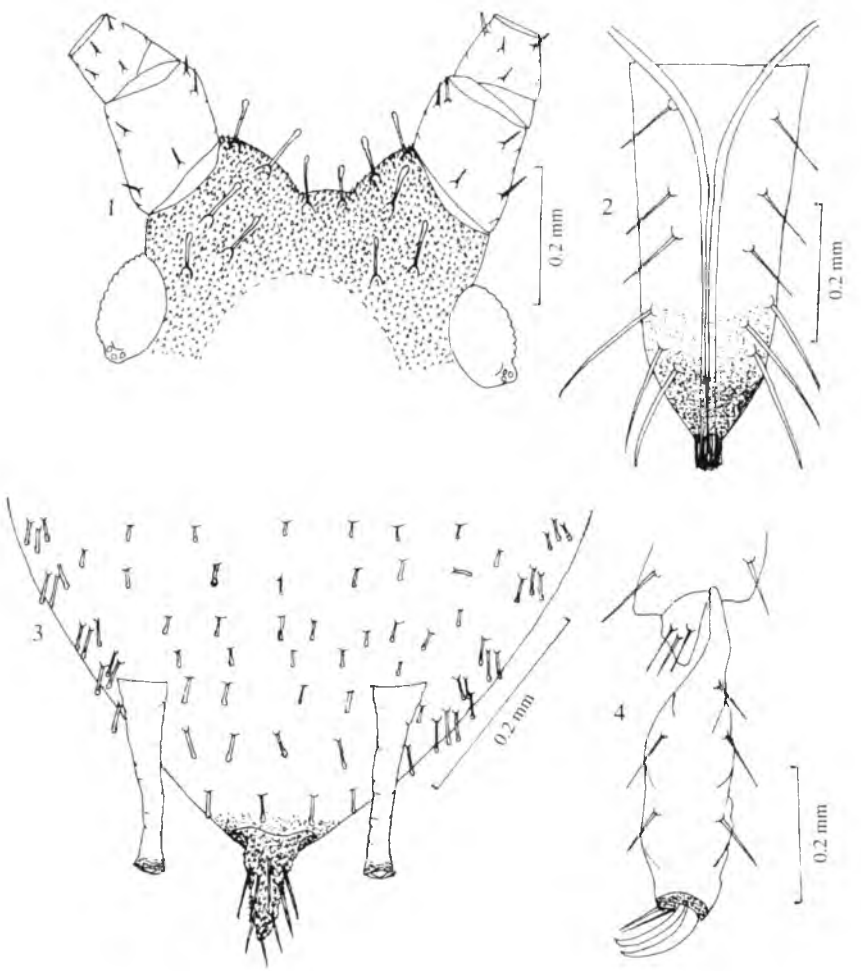


FIGURES 1–4: *Cryptomyzus elshotze* (Apterous viviparous female) 1. Head; 2. Ultimate rostaral segment; 3. Posterior part of abdomen; 4. Second joint of hind tarsus.

tergites 0.043–0.050 and 0.057–0.060 mm long and 1.30–1.50 and 1.70–1.80 times the b.d.III, respectively. Siphunculi long cylindrical, faintly imbricated bearing 2–3 inter connecting striae before flange, 0.20–0.21 times the body and 3.00–3.21 times the cauda. Cauda elongated with two constrictions bearing 6–8 hairs on the anterior margin. Legs in general pale brown, with tips of femora and tibiae which are brown, tarsi dark, femora and tibiae smooth, tarsi imbricated; F.TC. 3,3,3.

Measurements of holotype (mm)

Body length 2.76, width 1.30; antenna 3.42; antennal segments III : IV : V : VI :: 0.81 : 0.63 : 0.64 : (0.18 + 0.62); u.r.s. 0.18; h.t.2.0.12; siphunculus 0.56; cauda 0.18.

Holotype

Apterous viviparous female, India: Uttarpradesh; Garhwal Himalaya: Bhairbghati, 5.V.1994 from *Elshotzia* sp. (coll. D. Bhattacharya).

Paratypes

9 apterous viviparous females and 5 nymphs, collection data as in holotype.

Remarks

This species in having marginal hairs on anterior abdominal tergites arranged in groups of 3, cauda with 6–8 and 3 hairs on first tarsal segments comes closer to *Cryptomyzus korschelti* Börner (1938) and *Cryptomyzus taoi* Hille Ris Lambers (1965). However, it differs from the former in the shorter ratio of p.t. to base of segment VI (8–10 times in *korschelti*), less number of secondary hairs on u.r.s (14–18 in *korschelti*) and shorter ratio of siphunculus to cauda (4.60 in *korschelti*). It also differs from *taoi* in having shorter ratio of u.r.s to h.t.2 (2.75–2.38 in *taoi*), more number of secondary rhinaria on antennal segment III (8–11 in *taoi*) and shorter ratio of p.t. to antennal segment III (1.56–3.30 in *taoi*).

***Schoutedenia emblica andhraka* David and Lambers**

Cerciaphis emblica Patel and Kulkarni, 1953, *J. Bomb. Nat. Hist. Soc.*, **51**: 435.

Schoutedenia emblica andhraka, David and Lambers, 1956, *Indian J. Ent.*, **18**: 41.

Schoutedenia emblica Remaudiere, 1990. *Bull. Soc. Ent. Fr.* **94**: 9; Ghosh and Agarwal, 1993. *Fauna India* (Zool. Surv. India), **6**: 46; Bhattacharya and Dey, 1996. *Entomon*, **21(3&4)**: 289.

Apterous viviparous female

Body 1.70–1.75 mm in length and 0.91–0.94 mm as the maximum width. Head pale medium and lateral frontal tubercles absent; dorsum with 4 pairs of hairs; dorsal cephalic hairs short with blunt apies, longest one on vertex 0.013–0.016 mm long and 0.36–0.40 times the b.d.III. Antennae 5-segmented, 0.68–0.69 times the body length, segment I and II pale rest of the flagellum gradually become brown; flagellum imbricated, segment III without any secondary *rhinaria*; longest hair on segment III 0.023–0.027 mm long and 0.63–0.72 times as long as basal diameter of the segment III; processus terminals 0.23–0.27 times as long as base of last antennal segment. Rostrum reaches midcoxae, ultimate rostral segment little blunt, 0.94–1.00 times as long as second joint of hind tarsus and bears 2 secondary hairs, abdominal dorsum pale, membranous anterior tergites with 6–8 hairs, tergites 7 and 8 each with 4 and 2 hairs respectively. Longest hair on antenier tergites 0.36–0.46 times as long as b.d.III, these on 7th 0.63–0.72 times and on 8th 0.72–0.81 times as long as b.d.III, respectively. Siphunculi conical, broad at base 0.046–0.047 times the length of the

body and 0.134–0.138 times the width of head across the eye. Cauda oval with 2–4 hairs little brown, distal region of femora with imbrications. First tarsal segments with 3 hairs.

Measurement of one specimen (mm)

Length of body 1.70; width 0.91; antenna 1.14; antennal segments *III* : *IV* : *V* :: 0.39 : 0.21 : (0.18 + 0.08); u.r.s. 0.51; h.t.20.51, siphuculus 0.08.

Material studied

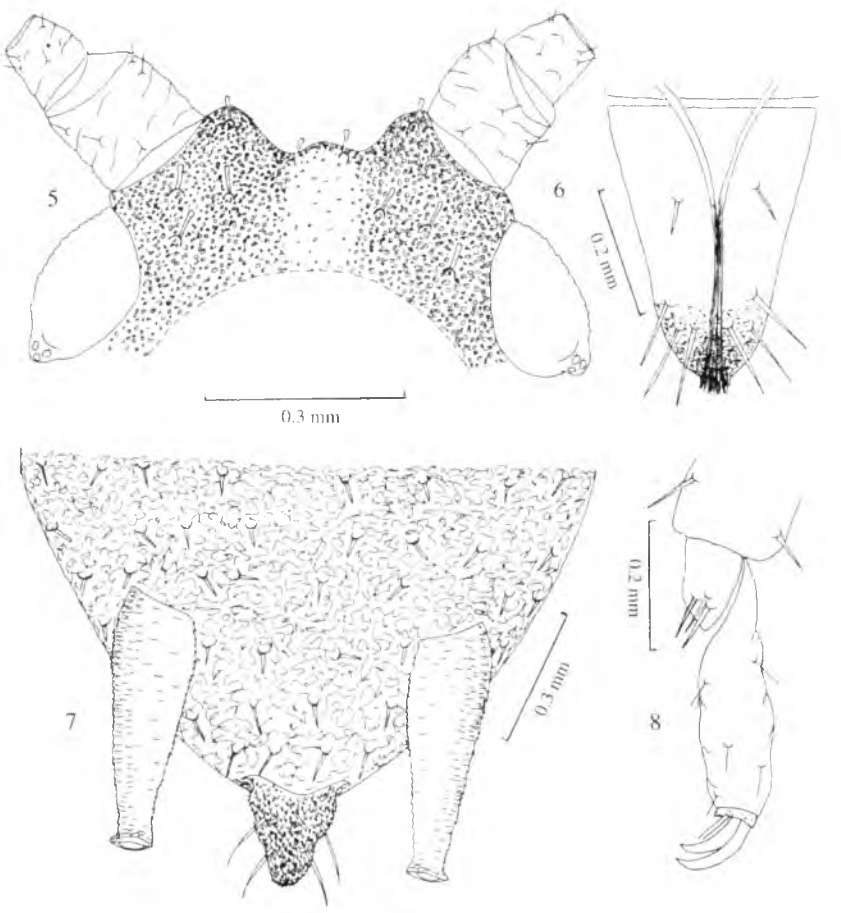
10 apterous vivaparous females and nymphs. India: Uttarpradesh: Gyansu (Garhwal Himalaya), 12.VI.1994 from *Spirea* sp. (Collector, S. R. Dey).

Remarks

Schoutedenia emblica was originally described by Patel and Kulkarni (1953) as *Cerciaphis emblica*. Later, David and Lambers (1956) treated the genera *Cerciaphis* Theobald and *Setaphis* v.d. Goot as synonym of *Schoutedenia* Rübsamen, at the same time they described the subspecies *S. emblica andhraka* and treated *S. emblica* as distinct species. They have differentiated their subspecies from *sensu, stricto*, on the basis of very small processus terminals, small number of secondary rhinaria in alatae. Eastop and Lambers (1976) listed *Cerciaphis* Theobald along with other related genera under *Schoutedenia* Rübsamen. Remaudiere (1990) during the revision of *Schoutedenia* considered *S. emblica* (Patel and Kulkarni) as valid without going to the necessity of establishing a new combination as made by David and Lambers (*op. cit.*) Ghosh and Agarwal (1993) put forward the same fact as made by Remaudiere (1990). In the present study the materials collected from Western Himalaya which revealed that the characters by which David and Lambers (*op. cit.*) established the subspecies is justified. Hence, the present authors concur with David and Lambers (*op. cit.*) and treat *Schoutedenia emblica andhraka* as valid one. A detailed description of the apterous viviparous female of the same is provided.

***Vesiculaphis polygonii* sp. nov. (Figs 5–8): Apterous viviparous female**

Body 1.64–1.67 mm wide. Head of 'Liosomaphidine' type as in pieridis group (Miyazaki, 1980), dorsum warty and corrugated leaving a central space almost free, venter warty along anterior and lateral margins, dorsum with 5 pairs of hairs with expended apices, longest one on vertex 0.013–0.016 mm long and 0.66–0.71 times the b.d.III. Antennae 6-segmented 0.072–0.073 times the body, pale brown except for segment I and II, apices of segment V and whole of the segment VI somewhat dark brown; segment I dorsally almost smooth but ventrally warty and with 5 hairs while segment II with few warts bearing 4 hairs; flagellum gradually imbricated apicad, longest hair on segment III 0.40–0.54 mm long and 0.50–0.57 times the b.d.III. p.t. 2.88–3.11 times the base of segment VI and 1.13–1.16 times the antennal segment III. Rostrum reaches the mid-coxae. Ultimate rostral segment 1.00–1.03 times the second



FIGURES 5–8: *Vesiculaphis polygoni* (Apterous viviparous female) 5. Head; 6. Ultimate rostaral segment; 7. Posterior part of abdomen; 8. Second joint of hind tarsus.

segment of hind tarsus and with 2 hairs. Thorax rugose midthoracic furca with separate arms. Abdomen dorsally densely corrugated, dorsum with short hairs arising from elevated bases and with expanded apices, anterior tergites with 8–9 hairs, longest one 0.010–0.011 mm along and 0.50–0.58 times b.d.III; tergites VII and VII each with 4 hairs; longest hairs on these tergites 0.011–0.013 mm and 0.013–0.016 mm long and 0.58–0.66 times and 0.66–0.83 times b.d.III. respectively. Siphunculi dark brown distally swollen, gradually, prominently warty apicad with 1 or 2 interconnecting striae before well developed flange, 0.19–0.21 times body and 2.00–2.40 times the cauda. Cauda pentagonal with 4 hairs. Venter of abdomen finely spinulose, subgenital plate with 2 hairs on the anterior margin and 14–15 hairs on the posterior margin. Legs pale except the tarsi which are brown; femora little scabrous specially on the distal half, tibiae smooth, tarsi poorly imbricated. F.T.C. 3, 3, 3.

Measurements of holotype (mm)

Body length 1.67, width 1.17; antenna 1.32, antennal segment *III* : *IV* : *V* : *VI* :: 0.32 : 0.21 : 0.13 : (0.12 + 0.37); u.r.s. 0.36; h.t.2. 0.35; siphunculus 0.32; cauda 0.13.

Holotype

Apterous viviparous female, India: Uttarpradesh: Garhwal, Lanka, 12. VIII. 1994, from *Polygonum alatum* (collector S. R. Dey).

Paratypes

9 apterous viviparous females and 3 nymphs, collection data as in holotype.

Remarks

Following Miyazaki (1980) this species having Liosomaphidine type of head belongs to pierids group of the genus *Vesiculaphis* del Guercio. Among the species under pieridis group this species comes close to *rhododendri* Ghosh and Raychaudhuri (1972) in having F.T.C. 3, 3, 3; u.r.s. with 2 secondary hairs and similar nature of siphunculi. But it differs from *rhododendri* in the longer ratio of p.t. to base of segment VI (1.00–1.30 in *rhododendri*); shorter ratio in u.r.s. and h.t.2. (1.50 in *rhododendri*) and longer ratio of antennae to the body (0.40–0.41 in *rhododendri*).

The type materials are deposited in the collection of Department of Zoology, University of Kalyani.

Abbreviations

Aptera/e = Apterous viviparous female/s; alata/e = Alate viviparous female/s; h.t.2 = second segment of hind tarsus; F.T.C = First Tarsal Chaetotaxy; p.t. = processus terminalis; b.d.III = basal diameter of antennal segment III; u.r.s = ultimate rostral segment.

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Species Diversity of Mosquitoes (Diptera: Culicidae) in Mangrove Ecosystem in South India

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ABSTRACT: Species diversity of mosquitoes in a mangrove ecosystem in India is reported for the first time. A total of 15 species belonging to 5 genera and 10 subgenera were recorded. Tree holes in *Avicennia marina* and *A. officianalis*, crab holes and swamp pools constituted the larval habitats. Habitat preference was evident as 5 species were found to breed in tree holes and 5 species in swamp pools without overlap of species in these habitats. *Aedes reginae* was predominant in tree holes and occurred throughout the year. Maximum number of species was found in tree holes between October–January when salinity was low due to high rainfall. Predominant species in swamp pools were *Cx. sitiens*, *Ae. portonovoensis* and *Ae. lugubris* and salinity influenced by rainfall governed the succession of these species. Adults were collected resting on aerial roots, in tree holes and crab holes and as biting females. *Ae. lugubris* fed viciously and formed the predominant species biting in the mangrove forest.

KEYWORDS: mosquito species diversity, Pichavaram, mangrove, tree hole, crab hole, swamp pool, salinity.

Most of the mosquito faunistic studies in India (Dhanpal and Naik, 1986; Nagpal and Sharma, 1987; Khamre and Khaliwal, 1988) have been done in relation to geographic locations. These studies provide information on the distribution of mosquito species in different regions or states. A more ecological approach, however, would be to conduct such faunistic studies with reference to a particular ecosystem. Among the many ecosystems, the mangrove forest is unique due to the edaphic and climatic factors as well as the typical flora. Although the Indian coastline extends to over 5700 km and the total mangrove area is estimated to be approximately 7,00,000 ha (Sidhu, 1963), there is no exclusive record of the mosquito species occurring in the mangroves. Mosquito species diversity, larval habitats, diversity pattern and species predominance in different larval habitats, and salinity levels as a determinant of species prevalence in

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larval habitats are reported for the first time from a mangrove forest in the southeastern coast of peninsular India.

The study site was Pichavaram mangrove forest, located in Tamil Nadu state on the southeast coast of India at latitude 11°27′ North and longitude 79°47′ East. It is an estuarine type of mangrove situated at the end of Uppanar canal which is a distributory of river Coleroon. The mangrove is connected to the Vellar estuary in the north and Coleroon estuary in the south by a well-developed backwater system and hence is traversed by large number of channels and creeks. The site has the special feature of being more or less completely separated from the sea by a narrow spit but the tidal rhythm is quite noticeable in the mangrove area. The mangrove is colonized by 14 exclusive mangrove species besides which 18 associate species have also been recorded (Selvam, 1996). Based on the distribution pattern of the flora, three zones have been identified, namely, Rhizophora zone, Avicennia zone and Suaeda zone. The Rhizophora zone occurs as a narrow strip along the tidal creeks and channels, which is immediately followed by the Avicennia zone.

The study was done from June 1997 to May 1998 by making monthly collection in four islets. During each visit four persons collected for five hours and the total time spent for mosquito collection during each survey was uniform throughout the study period. Both immatures and adults were collected. Immatures were collected from all types of larval habitats available in the area. The dipper was used for sampling swamp pools while pipette was used for tree holes. Wherever tree holes were deep, the commercially available plastic kerosene pump was used as a suction pump for siphoning out the water along with larvae. When water increased in tree hole it was filtered through a sieve and the larvae retained and transferred to containers. Larvae from crab holes were collected with pipette and when possible with small ladle. Crab holes were also dug up to gain access to the water when necessary. Resting adults were collected amongst vegetation, aerial roots, in tree holes and crab holes with an aspirator. Stationery direct bait catch (Service, 1976) was done to assess the biting density. Catches made for a period of 15 min each month was used as a unit measurement for determining the biting density.

Species identification was done following keys of Christophers (1933), Barraud (1934), Huang (1979), Reinert (1974), Sirivanakarn (1976) and Tanaka *et al.* (1979). Larvae were reared to adults and identification was based mainly on adult characters and wherever necessary associated larval and pupal skins were mounted for confirmation. Specimens of all species recorded are deposited in the mosquito museum at the Vector Control Research Centre, Pondicherry.

Salinity as chlorides was determined for samples collected from ten swamp pools, tree holes and crab holes during each month. Rainfall and temperature data of the study area for the period of study was obtained from the Centre of Advanced Study in Marine Biology, Parangipettai. Maximum rainfall (895.5 mm) was recorded in November with highest number (18) of rainy days. The mean maximum temperature ranged from 29.48 °C in November to 38.21 °C in June and mean minimum ranged

from 22.76 °C in November to 26.6 °C in June. Relative humidity was also highest (92%) in November and lowest (69%) in June.

Species diversity

Fifteen species belonging to 5 genera and 10 subgenera were recorded from a total of 8572 mosquitoes of which 6735 were collected as immatures and 1837 as adults.

Genus	Subgenus	Species (number collected)
Aedes	*Diceromyia*	*reginae* Edw. 1922 (2670)
Aedes	*Rhinoskusea*	*portonovoensis* Tewari and Hiriyan, 1991 (1759)
Aedes	*Stegomyia*	*albopictus* (Skuse) 1894 (4)
Aedes	*Stegomyia*	*krombeini* Huang, 1975 (57)
Aedes	*Verrallina*	*lugubris* Barraud, 1928 (1164)
Anopheles	*Cellia*	*subpictus* Grassi, 1899 (49)
Anopheles	*Cellia*	*vagus* Doenitz, 1902 (8)
Culex	*Culex*	*infula* Theo., 1901 (5)
Culex	*Culex*	*pseudovishnui* Colless, 1957 (1)
Culex	*Culex*	*sitiens* Wied. 1828 (1604)
Culex	*Culex*	*tritaeniorhynchus* Giles, 1901 (35)
Culex	*Culiciomyia*	*spathifurca* (Edw.) 1915 (908)
Culex	*Eumelanomyia*	*brevipalpis* (Giles) 1902 (306)
Mansonia	*Mansonioides*	*annulifera* (Theo.) 1901 (1)
Uranotaenia	*Uranotaenia*	*annandalei* Barraud, 1926 (1)

Of these, *Culex infula*, *Cx. pseudovishnui*, *Cx. tritaeniorhynchus*, *Mansonia annulifera* and *Uranotaenia annandalei* were collected only as adults.

Diversity pattern in larval habitats and species predominance

Tree holes, crab holes, and swamp pools were the three types of larval habitats in this mangrove. Immatures of *Ae. albopictus*, *Ae. krombeini*, *Ae. reginae*, *Cx. brevipalpis* and *Cx. spathifurca* were collected from tree holes while that of *Ae. lugubris*, *Ae. portonovoensis*, *An. subpictus*, *An. vagus* and *Cx. sitiens* were collected from swamp pools. *Ae. portonovoensis* was present in both swamp pools and crab holes. Among tree hole breeding species, *Ae. reginae* occurred throughout the year and was the predominant species accounting for 67.6% of the total larvae collected in this habitat (Fig. 1) while in swamp pools, *Cx. sitiens* accounted to over 75% of the different species collected during April–May and August–September (Fig. 2).

Salinity level as a determinant of species diversity in larval habitats

Mean value (mg/l) of salinity for different months ranged from 745 (December) to 18000 (April) in tree holes, 650 (November) to 30550 (August) in crab holes and 750 (November) to 30180 (September) in swamp pools. While the minimum values were comparable, the maximum values were significantly ($p < 0.05$) higher in crab holes and swamp pools, but between these two habitats there was no significant

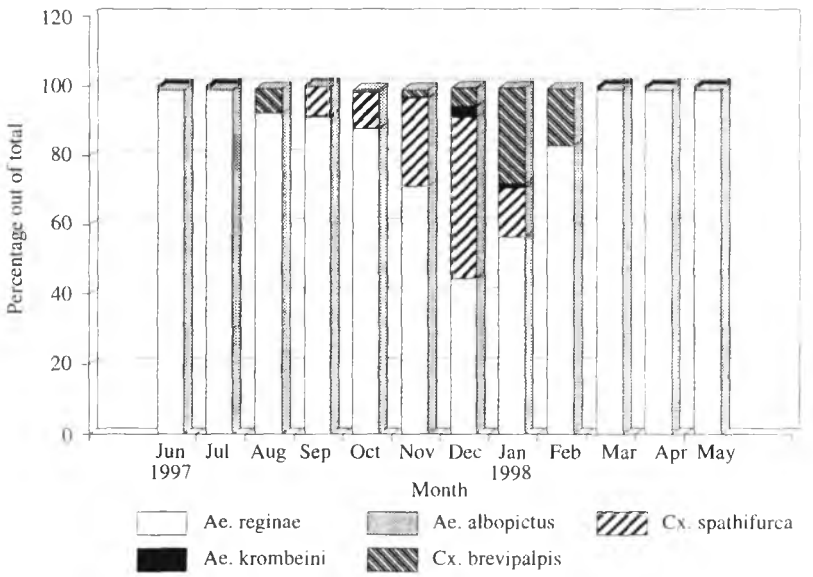

FIGURE 1. Species diversity and predominance in tree hole habitat.

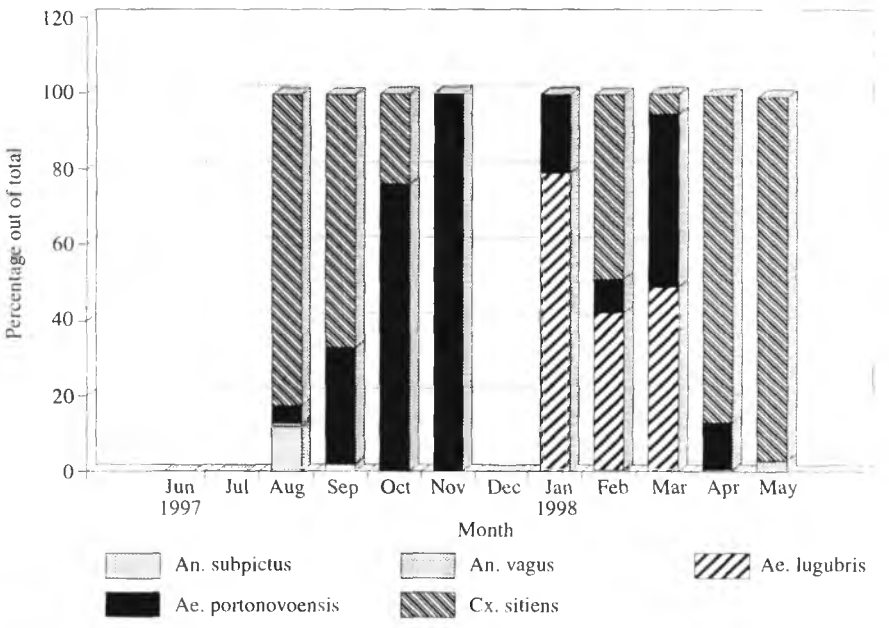

FIGURE 2. Species diversity and predominance in swamp pool habitat.

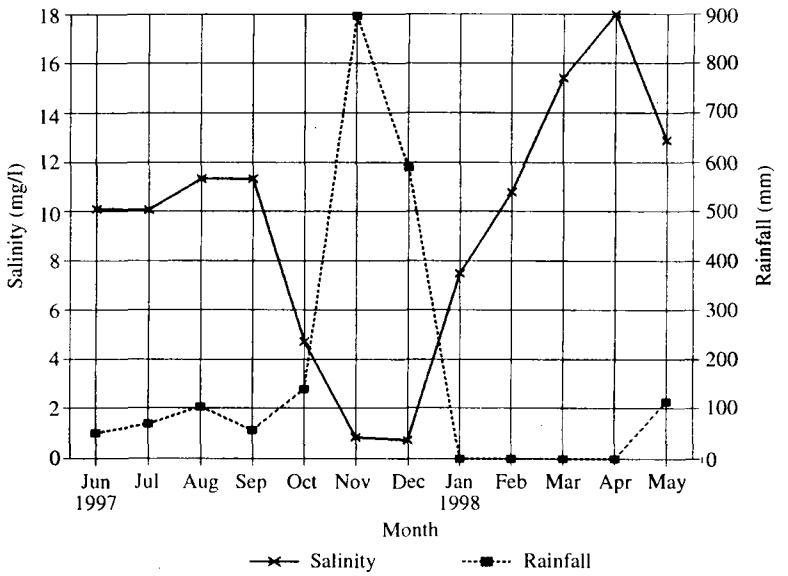

FIGURE 3. Relation between salinity in tree hole and rainfall.

difference ($t = 0.66$; $p = 0.525$). The relationship between salinity of tree holes and rainfall is shown in Fig. 3. Maximum number of species was found in tree holes between October–January (Fig. 1) when salinity was low due to high rainfall and only *Ae. reginae* was found throughout the year in spite of wide fluctuations in salinity. Species composition in swamp pools was also influenced by salinity fluctuations.

Adult prevalence

Adult resting sites comprised of crab holes, shrubs, tree holes and aerial roots of *Avicennia*. While *Ae. reginae* and *Cx. brevipalpis* were collected from tree holes and aerial roots, *Cx. sitiens*, *Cx. infula*, *Cx. pseudovishnui* and *Cx. tritaeniorhynchus* were found resting on aerial roots. *Ae. portonovoensis* and *Ur. annandalei* occurred in crab holes. *Ae. lugubris* was predominant among species that attacked man during day. Other species collected biting at the same period were *An. subpictus*, *Cx. infula*, *Cx. sitiens*, *Cx. tritaeniorhynchus*, *Ae. krombeini* and *Ae. reginae*. Seasonal biting incidence of *Ae. lugubris* determined by stationery direct bait catch is given in Fig. 4. Biting females attacked bait throughout the year but peak biting density occurred in November with another smaller peak in May.

In a mangrove swamp in Singapore, Laird (1988) found breeding of species belonging to only two genera, *Anopheles* and *Culex* while Ritchie and Jennings (1994) obtained only species of the genus *Aedes* in mangroves. The present record of 15 species belonging to 5 genera and 10 subgenera, which is the first report from a mangrove ecosystem in India, reflects the species diversity in the mangrove forest of

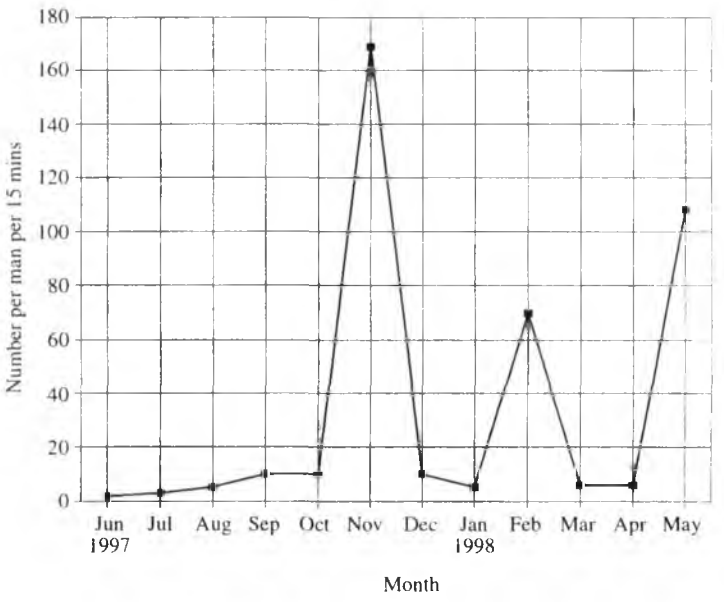

FIGURE 4. Seasonal biting incidence of *Aedes lugubris*.

Pichavaram. The record of *Ae. krombeini* in this area extends its range to the Eastern coast of India as it has been reported only in the Western hill ranges by Tewari *et al.* (1987). The occurrence of *Ma. annulifera* in the mangrove area is attributed to the *Eichhornia crassipes* flushed into the channels from the canal which is heavily infested with the hydrophyte.

Mangrove forest, being a brackish environment, mosquitoes that are considered as salt-water species are expected to colonize this ecosystem resulting in a restricted species diversity. Contrary to this, the present study has shown that mangrove forest can present a wider species diversity. This not only included salt water species like *Cx. sitiens*, but also fresh water species. *Cx. tritaeniorhynchus*, *Cx. pseudovishnui*, *Cx. infula*, *Ae. albopictus*, and *Ae. krombeini*. Their occurrence in this ecosystem gains significance, as most of these species are known disease vectors.

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Use of *Madhuca longifolia* (J. Koenig) Macbride seed oil in controlling pulse beetle *Callosobruchus maculatus* F. (Coleoptera: Bruchidae)

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ABSTRACT: Cowpea, *Vigna unguiculata* is an important pulse widely produced and consumed as a vegetable crop. *Callosobruchus maculatus* is a serious pest of stored cowpea. Experiments involved treatment of cowpea seeds with different doses of (0.25, 0.5, 1.0, 2.0 and 4.0 ml/100 g seeds) of *Madhuca longifolia* seed oil, neem oil (0.5 ml/100 g seeds) was used as positive control. Number of eggs laid and F1 adult emergence decreased in all the treatments. Application of *M. longifolia* oil at 0.5 ml/100 g of seeds is recommended as optimal dose to protect seeds without any damage. This does not affect the organoleptic properties and germination of the seeds. Utilization of *Madhuca* oil to protect cowpea seeds is ecofriendly and economically viable.

KEYWORDS: *Madhuca longifolia*, *Callosobruchus maculatus*, neem, cowpea.

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walpers is an important grain legume widely grown on small-scale farms. Pulse beetle, *Callosobruchus maculatus* causes enormous damage in stored cowpea seeds and many other pulses in tropical and subtropical world. Infestation starts even in the field. Adults lay eggs on immature pods and the larvae bore through the pericarp and feed within the developing seeds (Southgate, 1979). When such seeds are stored, the larvae continue to feed and eventually emerge as adults that cause secondary infestation (Talekar, 1988). Application of chemical insecticides and fumigants is not advisable as seeds are used for consumption (Talekar and Lin, 1992). Mixing plant oils with pulses is a traditional Indian method of protection against storage insect pests. Small amount of vegetable oil treatment does not affect the germination rate or flavour. Nutritive value of the seed is retained by the treatment when prepared for consumption (Hill and Schoonhoven, 1981a). Earlier works report the potentialities of vegetable oils in controlling storage pests (Hill and Schoonhoven, 1981b; Messina and Renwick, 1983; Pereira, 1983; Naik and Dumbre, 1984; Boughdad *et al.*, 1987; Ivbijaro, 1990; Su, 1991; Lienard *et al.*, 1993; Rajapakse

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TABLE 1. Number of eggs laid and adults emerged from cowpea seeds treated with *Madhuca* seed oil

Treatments	Dosages ml/100 g	Eggs laid	Adults emerged
Madhuca oil	0.25	26.4 ± 4.18^{f}	12.0 ± 1.58^{b}
	0.50	18.2 ± 3.35^{e}	0
	1.00	9.2 ± 3.49^{d}	0
	2.00	3.2 ± 1.92^{c}	0
	4.00	0	0
Neem oil	0.50	12.8 ± 1.9^{b}	0
Control	—	68.2 ± 5.39^{a}	44.8 ± 4.44^{a}

Mean ± SD
Within the column different alphabets was statistically significant ($p < 0.05$) by LSD

and Senanayake, 1997). Present work was undertaken to study the effectiveness of *Madhuca longifolia* seed oil on *C. maculatus* as well as to evaluate the effect of these oils on organoleptic properties and seed viability.

MATERIALS AND METHODS

Cowpea seeds, *Madhuca* oil and neem oil purchased from the local market were utilized for the experiments. Pulse beetle, *Callosobruchus maculatus* obtained from stock culture maintained at Entomology Research Institute, was used in the present investigation.

Effect of oil on egg laying and adult emergence

Madhuca oil at doses of 0.25, 0.5, 1.0, 2.0 and 4.0 ml was applied to 100 g cowpea seeds each, and shaken well to obtain uniform distribution of oils to the seeds. After treatment seeds were separated into five lots each having 50 seeds and stored in individual plastic containers. Two pairs of neonate adults were introduced in each container and covered with muslin cloth. Experiments were conducted at 28 ± 2°C and 70 ± 5% RH. Number of eggs laid on treated and control seeds were recorded after 15th day and all the dead and live insects were removed. After 30th day or when the first emergence starts, the number of F1 adults emerged from each treatment were recorded continuously till the last emergence (Approximately 30 to 40 days). Neem oil 0.5 ml was used as positive control. Five replicates were maintained for all the treatments. The data collected were subjected to Analysis of Variance (ANOVA) and the means were separated using Least Significance Difference test (LSD).

Organoleptic qualities of treated seeds

Cowpea seeds 200 g were treated with minimum effective concentration of *Madhuca* oil (0.5 ml/100 g seeds) (based on experiment), neem oil and control. After three months 100 g of treated seeds were drawn, washed thrice in tap water, soaked in

TABLE 2. Organoleptic test for the cooked cowpea seeds after 3-month storage period

Attribute	Control	Madhuca oil	Neem oil
Appearance	3	2	2
Texture	3	3	3
Taste	3	3	1
Flavour	4	3	1
Acceptability	4	3	0

0-very poor, 1-poor, 2-fair, 3-good, 4-very good

TABLE 3. Mean percentage of seed germination after oil treatment

Treatments	Mean % germination
Control	87.8 (69.60)^a
Madhuca oil	82.4 (65.23)^b
Neem oil	83.8 (66.40)^b

Within the column different alphabets was statistically significant ($p < 0.05$) by LSD.
Figures in parenthesis are arc sine transformed before ANOVA

distilled water for one hour and cooked in microoven for 20 minutes. Sufficient amount of salt was added before boiling the seeds. Seed appearance, taste, texture, flavour and acceptability were evaluated and determined.

Test for seed germination

Remaining 100 g of cowpea seeds were used for germination test. Randomly selected seeds 250 numbers were washed with tap water and soaked for 12 h in distilled water. Seeds were separated into 5 lots each having 50 seeds and placed in petridishes overlaid with wet cotton. It was stored in dark and after 48 h the germination of seeds was recorded. For control uninfested seeds were used. All data were transformed into arc sine values before subjecting to Analysis of Variance (ANOVA) and significant differences were marked using Least Significance Difference test (LSD).

RESULTS AND DISCUSSION

In *Madhuca* oil treatment, number of eggs laid by the beetle was decreased with increasing doses (Table 1). At higher dose (4.0 ml) *C. maculatus* failed to lay eggs on treated seeds. Significant differences were recorded between control and treatments. It is possible that oil deposits on the surface of the seeds might interfere with the movement and/or probing behaviour of ovipositing females. Possibly the repellent action and/or toxicity to adults would have reduced the egg laying capacity. Similar

mechanism of action was earlier reported by Singh *et al.* (1978), Messina and Renwick (1983) and Don Pedro (1989). F1 adult emergence was noticed only in the treatment with 0.25 ml/100 g seeds. The physical nature of the oil that block the oxygen supply for the developing embryo or due to the toxicity of some constituents of the oil used may be the reason for reduced egg laying and less emergence. Similar result was also reported by Credland (1992).

Both the oil treatment affected the appearance of the treated seeds. However, *Madhuca* oil did not affect the taste, flavour and texture of the seeds (Table 2). But neem oil treated seeds were unpleasant and bitter in taste. Van Huis (1991) reported that vegetable oils such as sesame, groundnut and sunflower oil do not affect the viability, palatability, cooking quality and physical appearance of seeds. In our studies also we found that *Madhuca/neem* oil did not affect the germination of treated seeds when compared to control germination is affected significantly (Table 3). It can therefore concluded that this oil can be used to protect cowpea seeds.

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Aspergillus tamarii (kita) a Potential Biocontrol Agent for Mulberry Leaf Roller, *Diaphania pulverulentalis* (Hampson) (Lepidopetra: Pyralidae)

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ABSTRACT: A field survey conducted for the incidence of mulberry leaf roller, *Diaphania pulverulentalis* has showed that it is more prevalent during June to February with maximum infestation during November. The micro flora of dead and abnormal larvae of *D. pulverulentalis* collected from field was analysed. The predominant flora was found to be *Aspergillus tamarii* which is also a pathogen on silkworm *Bombyx mori*. Cross infectivity test confirmed the pathogenicity, results showed that first instar larvae were highly suspectible to the infection of *A. tamarii than the later instars.*

KEYWORDS: *Aspergillus tamarii*, mulberry, biocontrol, *Diapharia pulverulentalis*

INTRODUCTION

Mulberry (*Morus* sp) is an essential food source of silkworm growth and development. Being Mulberry is a perrineal plant producing evergreen luxuriant foliage attracting various insects and non-insect pests. Among the pests, lepidopterans are the most severe and cause devastating damage to mulberry. Recently the leaf roller, *Diaphania pulverulentalis* inflicts severe damage in south India (Siddegowda *et al.*, 1995; Geetha Bai *et al.*, 1997; Rajadurai *et al.*, 1999). Where as mulberry pyralid *Gyphodes pyloalis* Walker was reported to be severe in northern states (Mathur, 1980; Sharma and Tara, 1985; Watanabe *et al.*, 1988) reported that *B. mori* DNV1, DNV2 and IFV were the common inhabitants of *G. pyloalis*. Takahashi *et al.* (1995) reported the occurrence of two epiphytic bacteria *Viz.*, *Pseudomonas syringae*. Van Hall, and *Erwinia herbicola*. Smith, on *G. pyloalis*, Walker, a pest of mulberry. In this study an attempt was made to identify the occurrence of entomopathogenic micro flora on the larvae of leaf roller and its detrimental effect was studied.

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TABLE 1. Occurrence of *Aspergillus tamarii* (kita) on mulberry leaf roller, *Diaphania pulverulentalis* during different months (Larvae examined $n = 80$)

Month	Percent infection
July 1999	0.00
August 1999	0.00
September 1999	11.25
October 1999	15.00
November 1999	26.25
December 1999	13.75
January 2000	2.50
February 2000	0.00
Mean±SD	8.6 ± 8.98

TABLE 2. Mortality of *Diaphania pulverulentalis* larvae due to *Aspergillus tamarii* (Kita)

Instars	Days after treatment**							***Per cent pupation	***Per cent moth emergence
	1	2	3	4	5	6	7		
I	5.45	17.31	14.86	8.26	0.86	0.25	0.25	0	0
	(2.44)	(4.22)	(3.92)	(2.96)	(1.17)	(0.87)	(0.87)	(0)	(0)
II	3.46	16.15	10.58	5.80	11.06	2.59	0.58	0	0
	(1.99)	(4.08)	(3.33)	(2.51)	(3.40)	(1.76)	(1.04)	(0)	(0)
III	1.09	2.63	13.26	7.06	1.09	0.00	0.25	18	8
	(1.20)	(1.77)	(3.71)	(2.75)	(1.26)	(0.70)	(0.87)	(24.83)	(16.35)
IV	0	0	2.53	8.50	6.95	0.25	0.25	67	34.66
	(0.70)	(0.70)	(1.74)	(3.00)	(2.73)	(0.87)	(0.87)	(55.13)	(36.10)
V	0	0	0	0	0	0	0	76.66	71.33
	(0.70)	(0.70)	(0.70)	(0.70)	(0.70)	(0.70)	(0.70)	(61.21)	(57.67)
F test	*	*	*	*	*	*	*	*	*
SEM±	0.35	0.38	0.22	0.19	0.15	0.14	0.15	2.39	1.30
CD 5%	1.12	1.22	0.72	0.63	0.49	0.43	0.48	7.560	4.70

*Significant at 5% level.
**Figures in Parenthesis are $\sqrt{x} + 0.5$ transformations.
***Figures in Parenthesis are angular transformed values

MATERIALS AND METHODS

A detailed survey on the incidence of leaf roller was made in the mulberry fields of Kanakapura Taluk, Bangalore Rural district during July 1999 to February 2000 and

TABLE 3. LT_{50} of mulberry leaf roller larvae due to *Aspergillus tamarii* (Kita)

Instar s	X^2	LT_{50}	LT_{99}	Factual limits		Regression equation $Y = a + bx$
				Upper	Lower	
I	2.92	0.92	4.39	1.19	0.61	$5.12 + 3.43x$
II	4.93	1.66	5.28	1.94	0.39	$3.97 + 4.63x$
III	9.66	3.75	8.57	4.15	3.26	$1.26 + 6.49x$
IV	5.58	5.43	11.34	5.94	4.89	$1.26 + 6.49x$
V	—	—	—	—	—	—

per cent infection was estimated. During the survey abnormal and dead larvae of leaf roller were collected from the fields and the micro flora present on them were isolated by placing the specimen directly on culture media plates containing nutrient agar(NA) for bacteria and Martins rose bengal streptomysin sulfate agar (MRBA) for fungi. Ten replicates of each were maintained separately for bacteria and fungi. After the growth and development of colonies they were identified using taxonomic key. After the incubation period, only few *Bacillus* colonies were noticed on nutrient agar plates. The predominant growth of fungi on MRBA were identified as per synoptic key and was found as *Aspergillus tamarii*. Koch postulate was verified by testing for the cross infectivity by inoculating the identified fungi to the healthy larva of leaf roller and confirmed for the disease occurrence.

The inoculation of the fungus *A. tamarii* to the healthy larvae of the leaf roller was made immediately after hatching and after moulting at a spore load of 6×10^5 conidia/ml by smearing the conidial solutions with fine brush thoroughly on both the surface of the larvae. The effect of *A. tamarii* on all the 5 instars were tested. Observations were recorded at 24 hrs interval for 7 consecutive days. Percent pupation and moth emergence in different instars were recorded. LT_{50} values were estimated in different instars of larva.

RESULTS AND DISCUSSION

The occurrence of entomopathogenic fungi present on mulberry leaf roller, *Diaphania pulverulentalis* during July 1999 to February 2000 was recorded and the per cent infection is presented in Table 1. The incidence was more prevalent during November followed by October and December. The findings of the survey is in accordance with the findings of survey made earlier by Rajadurai *et al.* (1999) in Karnataka, Andhra Pradesh and Tamilnadu where they had reported prevalence of leaf roller on mulberry from June to February in South India. Mortality of different instars of *D. pulverulentalis* due to *A. tamarii* at a conidial load of 6×10^5/ml differed significantly with instars (Table 2). First and second instar larvae were found highly susceptible to infection compared to later instars. In first instar larva maximum larval mortality was recorded on 2nd and 3rd day after inoculation (17.31 and 14.86/50 larvae). A similar

trend in mortality was also seen in second and third instar larvae whre the mortality was high during 2nd and 3rd day after inoculation. Pupation and moth emergence was not seen in 1st and 2nd instar larvae. As the larval stage advanced, resistance to *A. tamarii* was found and per cent pupation and moth emergence was maximum in V instar larvae (76.66 and 71.33) followed by IV (67 & 34.66). No mortality was observed in V instar but mortality was very less in IV instar which has clearly showed the development of resistance in older larva.

LT_{50} values were calculated at a conidial load of 6×10^5/ml and is presented in Table 3. The least LT_{50} value, 0.92 days, was recorded in 1st instar larvae indicating the maximum susceptibility followed by 2nd instar with an LT_{50} value of 1.66. LT_{50} values couldn't be calculated for V instar since the larval duration was only 2.35 days and no mortality was recorded during that period.

The result of this cross infectivity study clearly showed that *Aspergillus tamarii* which is a pathogen on *Bombyx mori* also caused disease on mulberry leaf roller. The finding also showed that *A. tamarii* can be effectively mass multiplied on culture media and inoculation can be used as a potential bio control agent for mulberry leaf roller specially in the early instar stages by spraying the conidial suspension. Utilization of *A. Tamarii* (Kita) as a potential bio-control agent of mulberry leaf roller, *D. pulverulentalis* is a first report under laboratory studies.

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Biology of *Phanerotoma noyesi* Zettel (Hymenoptera: Braconidae), a Parasitoid of the Leaf-roller Pest of Mulberry, *Diaphania pulverulentalis* (Hampson) (Lepidoptera: Pyralidae)

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ABSTRACT: *Phanerotoma noyesi* Zettel (Hymenoptera: Braconidae) is a solitary, arrhenotokous, egg-larval, endo-parasitoid of *Diaphania pulverulentalis* (Hampson) (Lepidoptera: Pyralidae). Studies on the biology of *P. noyesi* were conducted in the laboratory at 25 ± 1 °C RH 70–80% and are reported here. The duration of egg stage was 21–24 hrs. There were three larval instars: the duration of I, II and III instars were 8–9, 1–2 and 3–4 days, respectively. The pre-pupal period was two days and pupal period was 7–8 days. The mean developmental period from egg to adult was 22–26 days. There was no pre-oviposition period. The sex-ratio was 1:1.84 (male:female). Adult longevity was studied using five types of diets. Adult longevity was similar for mated male and female *P. noyesi*.

KEYWORDS: *Phanerotoma noyesi*, leaf-roller, *Diaphania pulverulentalis*.

INTRODUCTION

Phanerotoma noyesi Zettel (Hymenoptera: Braconidae) is a solitary, arrhenotokous, egg-larval, endo-parasitoid of the leaf-roller pest, *Diaphania pulverulentalis* (Hampson), a severe pest of mulberry (Geetha Bai *et al.*, 1997). This parasitoid has been recorded earlier from pod borer, *Tephrosia purpurea* (Zettel, 1990). Studies on development, mating, oviposition, sex-ratio and adult longevity were conducted and are presented here.

MATERIALS AND METHODS

Parasitoid culture

Field-collected *D. pulverulentalis* caterpillars were held in polythene bags (37 × 25 cm) and were fed on tender mulberry leaves. *P. noyesi* cocoons reared from these

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caterpillars were isolated in glass vials (15 × 15 cm) provided with cotton plug. When adult parasitoids emerged, they were fed on 50% honey solution and were allowed to mate.

To obtain host eggs, 25 to 30 *D. pulverulentalis* moths were enclosed in a potted plant, using nylon net. After oviposition, mulberry branches from these potted plants with host eggs on the leaves were provided to 3 days old 5 mated, gravid parasitoids for 24 hrs in a polythene bag (37 × 25 cm), provided with holes for aeration. Uninfested tender mulberry leaves were provided on alternate days to developing host caterpillars obtained from eggs exposed to the parasitoids, until fully grown parasitoid larvae emerged and pupated. The parasitoid cocoons were collected and were held in glass vials until adults emerged. The parasitoid culture was maintained at 25 ± 1 °C and RH 70–80%.

Life-history and morphology of immature stages

Experiments on the life-history of *P. noyesi* were conducted at 25 ± 1 °C and RH 70–80%. To study the development of the parasitoid, 3-days-old, 5 mated gravid parasitoids from the stock culture were released into a polythene bag with freshly laid *D. pulverulentalis* eggs on mulberry leaves. These females were removed after 2 hrs, and this was replicated five times. The host was dissected at different intervals and the developing parasitoid was examined. To study the incubation period of parasitoid eggs, infested host eggs were dissected 12 hrs after the host-parasitoid contact, under steriomicroscope, until hatching was recorded. The larval instars were determined by studying the shape and size of mandibles at different periods, by clearing larvae using Sinton's fluid (Chloral hydrate, 2 parts: Phenol, 1 part; Lactic acid, 1 part) (Geetha Bai, 1980) or by boiling larvae in 10% potassium hydroxide (Peter and David, 1992). They were washed in water, mounted on a slide and were observed under compound microscope. To study the sex-ratio of progeny, a gravid female was provided with host eggs daily, from the first day till its death and the sex-ratio of the progeny was recorded daily. These observations were replicated five times. Longevity of 10 mated male and female *P. noyesi* was studied by providing them daily with 50% honey, sucrose, glucose, or jaggery solutions, mucilage from *Hibiscus* flowers or water. Another set of parasitoids were not provided with any type of food. Their mortality was recorded daily.

RESULTS AND DISCUSSION

P. noyesi is a solitary parasitoid. Generally only one egg is laid within the host. When host availability is limited it resorts to superparasitism. It is an arrhenotokous species, as only male parasitoids develop from hosts parasitised by unmated females.

Immature stages

The size and duration of immature stages is furnished in table 1.

TABLE 1. Size and immature duration of *Phanerotoma noyesi*.

Sl. No	Stage	Length (mm) $\bar{X} \pm$ S.E.	Width (mm) $\bar{X} \pm$ S.E.	Duration
1	Egg	0.16 ± 0.00	0.06 ± 0.00	21–24 hrs
	Larva			
2	I instar	0.51 ± 0.19	0.11 ± 0.05	8–9 days
3	II instar	1.60 ± 0.34	0.39 ± 0.13	1–2 days
4	III instar	3.73 ± 1.32	1.11 ± 0.33	3–4 days
5	Pre-pupa	4.45 ± 0.04	1.38 ± 0.18	2 days
6	Pupa	4.49 ± 0.19	1.47 ± 0.13	7–8 days

$\bar{X}$ = Mean.
S.E. = Standard Error.

Egg

The egg of *P. noyesi* is oval in shape and is translucent. The chorion is thin and elastic. The parasitoid egg is laid inside the host egg, outside the host embryo, between the egg chorion and the host embryo. A freshly laid egg measures an average of 0.16 mm in length and 0.06 mm in width. The egg enlarges as development of the embryo proceeds. The incubation period is 21 to 24 hrs. After egg hatching the parasitoid larva enters the body of the host embryo, while still within the host egg. Generally only one egg is laid in a host egg.

Larvae

The first instar parasitoid larva is found within the host embryo for the first two days. After hatching of host egg, the parasitoid larva is found within the caterpillar. The larva is translucent and elongated. The head is triangular in shape. There are eight body segments besides the head and caudal segments. The mean length of first instar larva is 0.51 mm and maximum width is 0.11 mm. The mandible is 'comma' shaped and the sharp end tapers to a very fine point. The duration of first instar larva is 8 to 9 days. In case of *P. hendecasisella* the duration of first instar larva is 9 to 13 days (Peter and David, 1992).

The second instar larva, found inside the host caterpillar, has 12 body segments besides the head and caudal segments [Fig. 1(a)]. Its average length is 1.60 mm and maximum width is 0.39 mm. The duration of second instar larva is 1 to 2 days. Peter and David (1992) reported the duration of second instar larvae of *P. hendecasisella* to be 1 to 3 days.

The third instar larva has 12 body segments, besides the head and caudal segments. The caudal segment is black and is surrounded by a thin transparent body wall. It measures an average of 3.73 mm in length and 1.11 mm in maximum width. The duration of third instar larva is 3 to 4 days. In case of *P. hendecasisella* third instar

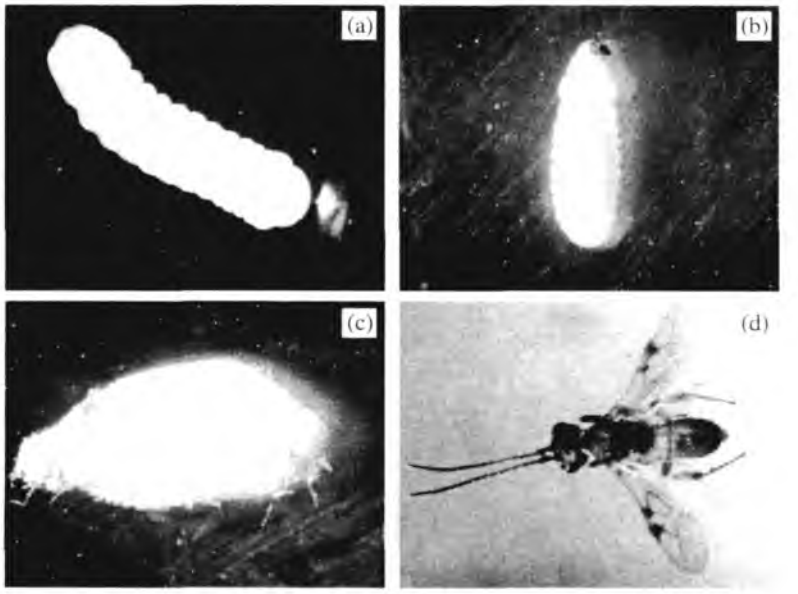

FIGURE 1. Immature stages and adult *Phanerotoma noyesi*. (a) Second instar larva, (b) Pre-pupa, (c) Cocoon, (d) Adult female.

larval duration is reported to be 2 to 3 days (Peter and David, 1992). It is interesting to note that larval duration of first instar is longest, followed by that of third instar and duration of second instar is shortest. A similar trend has been reported in *P. hendecasisella* also (Peter and David, 1992). Mature third instar larva pierces through body wall of the host caterpillar, discharges the black meconium and spins a cocoon around itself on the lower side of mulberry leaf and transforms into a pre-pupa.

Pre-pupa

The pre-pupa found inside the cocoon is creamish white in color after discharge of meconium [Fig. 1(b)]. The mean length of pre-pupa is 4.45 mm and maximum mean width is 1.38 mm. The duration of pre-pupa is 2 days. Peter and David (1992) have reported the duration of pre-pupa of *P. hendecasisella* to be 1 to 3 days.

Cocoon

The cocoon is silvery white in color and cylindrical in shape [Fig. 1(c)]. It is loosely spun. The average length is 5.83 mm and width is 2.43 mm.

Pupa

The pupa is initially creamish yellow in color and the three distinct regions of the body, the head, thorax and abdomen are formed. On the first day two antennal buds, three

ocelli and two pinkish compound eyes appear on the head. Three pairs of legs appear on the thorax. On the second day, mouth parts, such as labial palps, mandibles and maxillae appear and the segments on the abdomen are formed. On the third day, the body color is creamish brown, and eyes turn black. On the fourth day, development of wing buds begin. On the fifth day, most of the appendages are fully formed. Body color changes gradually to yellowish brown. The mean length of the pupa is 4.49 mm and maximum width is 1.47 mm. Duration of pupal stage is 7 to 8 days. Adult parasitoids emerge by piercing through the cocoon. Development is completed in 22 to 26 days.

Adult

Adult parasitoids are yellowish brown in color [Fig. 1(d)]. Females have a prominent ovipositor, which helps to distinguish them from males. Females are slightly larger than males. The mean length of male is 4.22 mm and width is 0.95 mm, and that of female is 4.34 mm and 1.14 mm, respectively.

Mating

Mating occurs soon after emergence of females. When a male comes in contact with a female, the male vibrates its wings for 3 to 4 seconds, and mounts a receptive female and engages the genital organs. The female remains still and erects its antennae. Mating lasts for 10 to 14 s (Av. = 11.4 s).

Oviposition

There is no pre-oviposition period. Oviposition commences on the day of emergence and continues until death. Normally only one egg is laid in a host egg. When there is a shortage of host eggs, superparasitism is observed, and 2 to 5 eggs are laid in the same host. Only one parasitoid completes its development and the supernumeraries are eliminated.

Sex-ratio

Sex-ratio of progeny recorded from mated females reared in the laboratory was 1:1.84 (male:female).

Adult longevity

Longevity of mated males and females fed on different diets is shown in Figs 2 and 3. Parasitoids fed on glucose lived for the longest duration, followed by sucrose, honey and jaggery. Those given mucilage from *Hibiscus* flowers, water and no food lived for a short duration. Longevity of mated males and females *P. noyesi* ranged from 3–72 days. Mari and Peydro (1992) have reported the longevity of *P. ocularis* Kohl to be 54.21 days. Peter and David (1992) reported a longevity of 6–22 days in case of female and 4–16 days in case of male *P. hendecasisella*, when fed on 20% honey solution.

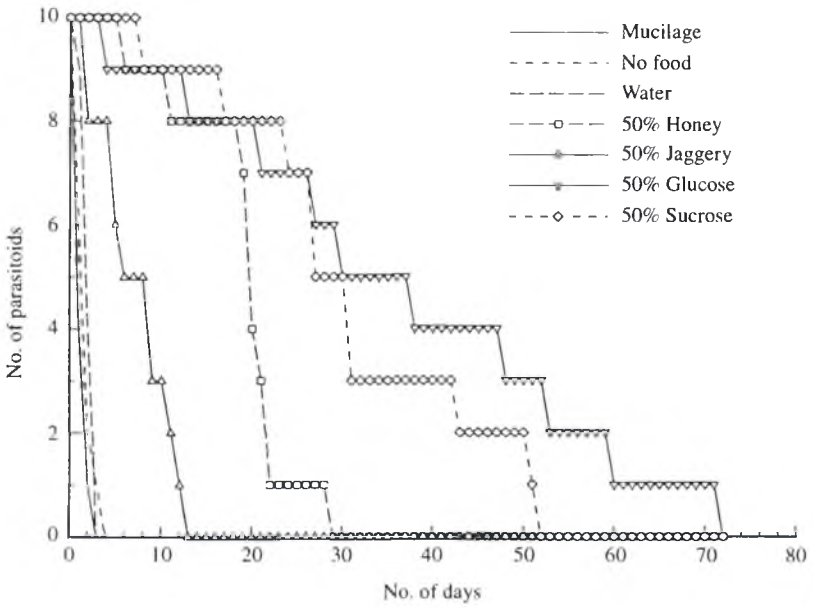

FIGURE 2. Longevity of mated-male *P. noyesi*

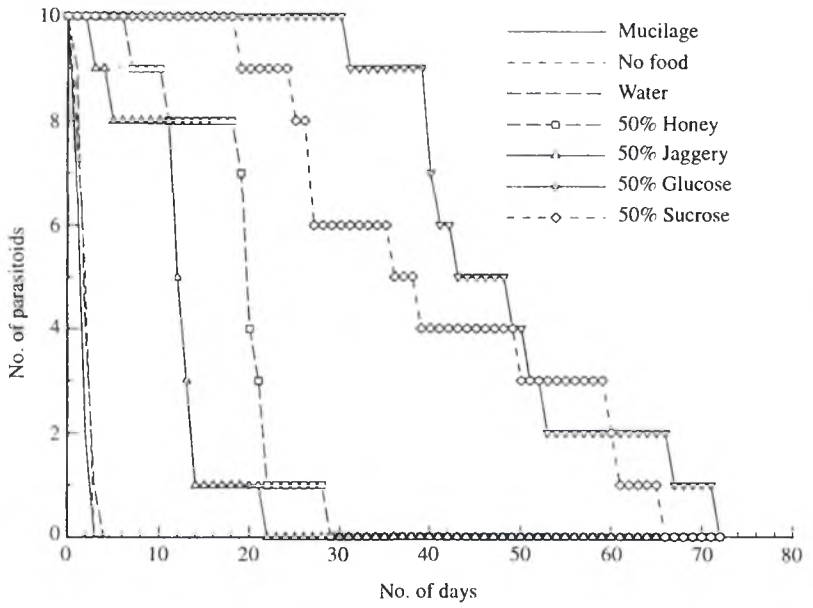

FIGURE 3. Longevity of mated-female *P. noyesi*

Very limited work has so far been done on *Phanerotoma* spp. Studies on the biology of *P. bennetti* Mues., a parasitoid of *Ancylostomia stercorea* (Zell.) (Lepidoptera: Pyralidae) in Trinidad (Bennett, 1960) and *P. hendecasisella*, a parasitoid of *D. indica* in India (Peter and David, 1992) have been recorded. There are similarities between *P. noyesi* and the two species studied earlier. *P. planifrons* Nees has been recorded from the mulbery moth, *Diaphania (Margaronia)* pyloalis from Japan (Watanabe, 1939) and China (Ding, 1984).

P. noyesi is the most predominant parasitoid of the leaf-roller pest in Karnataka, with a maximum parasitism of 67% (Anonymous, 1997). There appears to be scope for utilising *P. noyesi* as a biological control agent for management of the leaf-roller pest, in an integrated pest management programme, and this needs to be investigated. Endosulfan and fenvalerate are reported to have a low impact on *Phanerotoma*, a parasitoid of jasmine budworm, *Hendecacis duplifascialis* (Hampson), when the insecticides were used for management of jasmine budworm (Chandramohan and Manoharan, 1990). *P. ocuralis* is reported to have been released to combat the carobmoth, *Ectomyelois ceratoniae* Zeller on dates in an experiment in Tunisia (Khoualdia *et al.*, 1996). Further studies on *P. noyesi* are in progress.

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Culture and Rearing of *Aceria guerreronis* and its Predators

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ABSTRACT: Techniques for laboratory culturing and rearing of the coconut mite, *Aceria guerreronis* and few of its predators have been developed. Life history of *A. guerreronis* at 28 ± 1 °C and 70–75% RH has been traced and the duration of the egg, active and quiescent periods of the nymphal stages have been studied. The mite requires 8–10.5 days for the completion of development from egg to adult stage. Preliminary observation on predatory activities of the ant *Flagiolepis* sp., thrips *Tubulifera* spp., staphylinid beetle *Oligota* sp. and the mites *Amblyseius* spp., *Typhlodromus* sp. and a cheyletid species has been made. Among these, cheyletid mite appears to be a potential predator of *A. guerreronis*.

KEYWORDS: coconut, *Aceria guerreronis*, Predators, culture methods

INTRODUCTION

Coconut mite *Aceria guerreronis* has a history as a pest of coconut since two decades (Hall and Espinosa, 1981). The mite since its detection and description from the Guerrero state of Mexico (Keifer, 1965) has been found invaded several countries of the world (Hall *et al.*, 1980; Mariau, 1986; Haq, 2000; Haq *et al.*, 2000). Initial colonization of nut by of *A. guerreronis* requires only one gravid female, the arrival of which on the button is sufficient to establish population to induce further infection to other nuts of the inflorescence (Moore and Alexander, 1987). Chances of infestation by *A. guerreronis* can be correlated to the accessibility of space under the bracts (Moore, 1986) as the mite exhibits preference to fertilized flowers and avoids unfertilized flowers. The absence of mites on unfertilized flowers can better be considered as a reflection of the tight adpression of the bracts on the nuts (Julia and Mariau, 1979; Hall and Espinosa, 1981). Once the mite has invaded an inflorescence of a coconut, it can spread to adjacent ones through continuous migration by walking. Rapid spread from plantation to plantation and coconut belts is mainly performed through wind (Julia and Mariau, 1979; Griffiths, 1984; Haq, 2000). Recently, the mite has established the status of a serious pest of coconut in

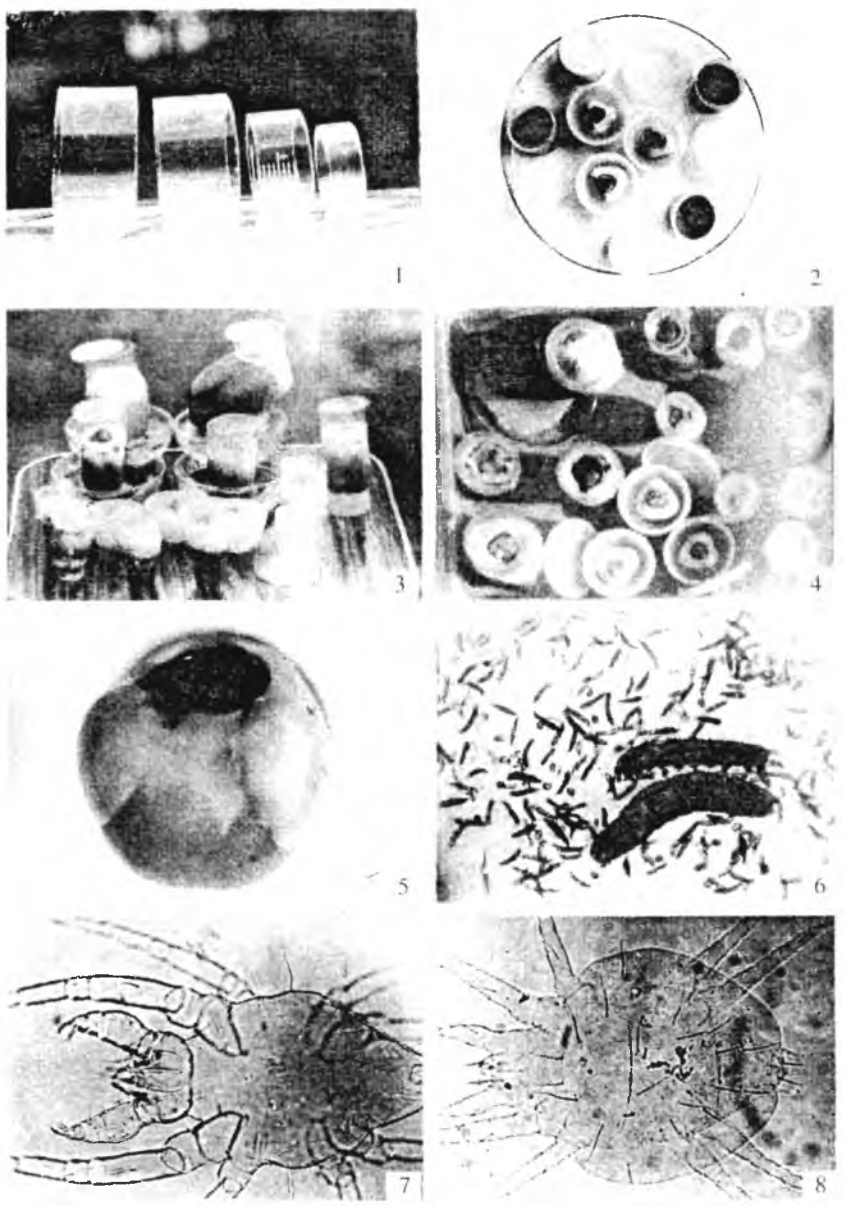
Kerala, other states of peninsular India and neighbouring islands (Haq, 1999a,b). Rapid invasion, colonisation and extensive damage caused by this mite in the coconut belts of India have created serious concern across the society. This has prompted the government to initiate control strategies on emergency basis. Accordingly, much effort has been made for controlling this pest. However, an effective method of regulation of the mite has not been achieved so far. Probably, lack of knowledge on some of the crucial aspects of the problem might have been instrumental for the unsatisfactory outcome of the measures adopted so far for the regulation of the pest. Several aspects in this regard need to be scrutinised in greater detail of which landing schedule of the mite on new buttons, mechanism of transmission, strategies of dispersal, feeding efficacy, pathogenic involvement, breeding potential, varietal susceptibility, influence of predators etc. are few deserving areas of priority. Analysis of these processes would provide better understanding of the intricacies of the problem, which may facilitate derivation of control strategies. This can be accomplished only through culturing and rearing of the mite successfully under laboratory conditions. Regretfully, our knowledge on culturing and rearing of *A. guerreronis* is very much limited. Taking into consideration of this lacuna, steps have been taken to develop suitable culturing and rearing techniques for *A. guerreronis* and its predators.

MATERIALS AND METHODS

Laboratory maintenance of *A. guerreronis* and its predators

Biological studies of *A. guerreronis* have successfully been carried out under laboratory conditions by developing appropriate culturing and rearing techniques. The culture techniques are mainly intended to develop suitable culture vessels. Various culture vessels developed here can be categorised into three types. They are: (1) cultures rings for rearing *A. guerreronis* (Fig. 1). (2) Plaster of Paris—charcoal based culture vessels for rearing predatory mites (Fig. 2) and (3) Culture bowls for rearing both the pest and predator (Figs 3 and 4). These culture vessels are made up of Borosil or Perspex glass for better penetration of light and easy handling of immature and adult stages. Commercially available Borosil glass rods of 5 cm diameter are bored and cut as rings with internal diameter of 2.5, 3, 3.5 and 4 cm with a height of 1.5–2.5 cm and thickness of 0.5–1 cm. Cover glasses of 1 mm thickness are suitably cut and used as lids for the culture vessels. These culture rings are fixed appropriately at the meristematic zone of the nut with the help of plasticine or paraffin wax. After introducing the mites, a drop of water is placed at the mouth of the culture rings to secure the cover glass in position. This arrangement considerably helps in observing the behaviour of individual stages of the mite through the cover glass and their manipulation according to need.

Predatory mites and other insects being very active are cultured in special plastic chambers of 3 cm diameter and 4 cm height with lid. These chambers are half filled with Plaster of Paris—animal charcoal mixture in the ratio 5 : 1. Fresh buttons are embedded in these culture vessels to which known number of *A. guerreronis* are released along with the predator for observing their feeding habits and breeding biology. A drop of 2% thymol solution and mycostatin suspension are also added to



FIGURES 1–8: 1: Glass rings of different size used for culturing *A. guerreronis* in the laboratory. 2: Culture vessels based with Plaster of Paris–charcoal mixture used for rearing predatory mites. 3: A view of the various types of culture vessels, rings and bowls used for rearing and culturing of mites. 4: Small open bowls used for rearing *A. guerreronis* and its predators. 5: Nut bearing triangular patches selected for the preparation of cultures. 6: Insect larval predators of *A. guerreronis*. 7: A male of *Cheyletus cocos* sp. nov. 8: A female of *Cheyletus cocos* sp. nov.

the culture base as bacteriostatic and fungistatic agents. Several such culture vessels are prepared for rearing of the predatory mites. A glass bowl of 3.5 cm diameter and 8.5 cm height is found excellent for rearing *A. guerreronis* and its predators. Coconut buttons of suitable age group are selected and fixed on plastic rings. The arrangement is placed in the centre of the bowl. The bowl is then filled with water just the level of the meristematic zone. Mites released at the meristematic zone are thus forced to remain there. Care is taken to retain the water level just the meristematic zone so as to prevent the movement of the mites away from the meristematic zone. For conducting feeding test of predatory mites, sufficient colonies of *A. guerreronis* would become a mandatory requirement in the laboratory. This can be achieved by collecting mite infested coconut inflorescence of 4–8 weeks. The peduncle of such inflorescence is kept in moistened condition in running water. An inflorescence can be kept as stock culture for 1–2 weeks in this way from which mites are collected for feeding experiments of the predatory mites in the laboratory.

RESULTS AND DISCUSSION

Development at biology of *A. guerreronis*

Gravid females of *A. guerreronis* invade the floral bracts when the coconut buttons reach the age of 3–6 weeks. On entering the buttons, the mites appear to crawl into the growing meristematic tissue under the perianth. Active feeding of the cell contents induces oviposition of the mite.

Eggs

Eggs are small, round (appear oval during later days) and glittering. These eggs are laid solitarily one after another and glued to the substratum with the sticky fluid discharged. Apart from the region of meristematic tissue, eggs are also found scattered on the inner portions and in between tepals also.

Hatching

Fresh eggs are transparent which turn milky white on incubation. Prior to hatching, a small projection develops at the animal pole where a slit appears longitudinally. Through the slit the anterior portion of the body comes out first. Subsequent wriggling movements of the nymph slowly emerge it out of the shell. The entire process of hatching needs about half an hour. The incubation period seems to range from 3–3.5 days.

Nymphal stages

First numph

The newly formed first numph is very small, sluggish and transparent. Within few minutes, the first nymph initiates feeding activity which lasts for 1.5–2 days at the end of which it becomes inactive. The body later becomes swollen and some what spindle shaped. Inactive period lasts for 1–1.5 days. Then it moults into the second nymph.

Second nymph

This stage is pale white in colour, elongated and vermiform. It is longer, more active and voraciously feeds on the cell sap. The active feeding period extends for 2–2.5 days and then it moults into the adult.

Quiescent stages

There are two quiescent phases, one between the first and second nymphs and the other between the second nymph and the adult. This period is represented by the inactive motionless phase. During this phase, the body enlarges and becomes, swollen, turgid and milky in appearance. This phase is terminated by moulting.

Moulting

This is a regular feature among mites and is initiated after the completion of an active feeding period and subsequent nymphal phase. A longitudinal slit appears at the anterior end of the body through which the gnathosoma and legs protrude out. The slit gets further widened by constant movements of the legs and the body resulting in the emergence of the new instar, leaving behind the moulting skin. Moulting skin appears to be broken into pieces on later observation. The post-embryonic development of the mite is completed within a period of 8–10.5 days at an average temperature of 28 ± 1 °C and RH of 70–75% in the laboratory (Table 1).

Natural predators A. guerreronis

A search on natural enemies of *A. guerreronis* has indicated predators comprising insects and mites as potential groups, with prospects in practical utilisation for biocontrol of the pest. The insect predators commonly found on nuts infested with *A. guerreronis* (Fig. 5) include thrips, ants, staphylinids, and several larval stages (Fig. 6). Of these, the ant belonging to *Flagiolepis* is found actively feeding on all stages of the mite which come out through the tepals of the nut. Two species of thrips belonging to *Tublifera* are found as voracious feeders of *A. guerreronis* both in field and laboratory conditions. In the laboratory, thrips complete development solely on food item comprising different stages of *A. guerreronis*. The staphylinid predator of *A. guerreronis* detected during field study belongs to *Oligota* and studies on its feeding efficacy and developmental biology are in progress.

The acarine predators collected during the study belong to the families Phytoseiidae and Cheyletidae. Of these, Phytoseiidae includes members of genera *Amblyseius* and *Typhlodromus*. The feeding potential of 3 species of *Amblyseius* appears to be quite encouraging both in field and laboratory conditions. When preliminary observations are made in culture rings in the laboratory, all the 3 species are found completing their development successfully on a food comprising *A. guerreronis*. A similar result has been obtained in the case of *Typhlodromus* sp. also. The predatory role of two cheyletid mites namely *Cheyletus cocos* sp. nov. (Figs 7 and 8) and *Cheletogenes* sp. appear to be remarkable and all the life stages of the former species of mite voraciously feed on

A. guerreronis both in the laboratory and in the field. Individuals of this species are often found crawling beneath the tepals through their broken edges and entering the meristematic zone of the nuts. The mite has been found often probing *A. guerreronis* and inserts its specially modified pedipalp into the body of the prey. A single mite can consume 190 to 220 individuals/eggs of *A. guerreronis* per day. After feeding, scar like remnants of the prey mites are left behind by the predator in laboratory cultures.

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Functional Diversity of PBAN in Castor Semilooper *Achaea janata* Linn. (Lepidoptera: Noctuidae)

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ABSTRACT: Members of PBAN-family of neuropeptides were found to control pheromone biosynthesis in adults, culticular melanization in larvae, myotropic activities and embryonic diapause in several insect species. Larval nervous system and different regions of gut in *Achaea janata* reacted positively to antiserum raised against Hez-PBAN. The findings suggest that the same neurohormone may be involved in different physiological events according to developmental stages.

KEYWORDS: *Achaea janata*, immunocytochemisty, larval nervous system, gut.

In many lepidopteran insects pheromone biosynthesis is regulated by a neurohormone viz. Pheromone Biosynthesis Activating Neuropeptide-PBAN (Raina and Kempe, 1992). The PBAN in *Helicoverpa zea* (Hez-PBAN) is a 33-amino acid peptide having an amidated carboxy terminus (Raina *et al.*, 1989). Cloning and sequencing of the Hez-PBAN cDNA revealed the preprohormone structure of precursor protein (Ma *et al.*, 1994). Besides the regulation of pheromone biosynthesis, peptide sequences encoded in prepro-Hez-PBAN appear to serve multiplie physiological functions. PBANs are members of the pyrokinin family of neuropeptides which are engaged in multiple physiological functions in insects characterized by a common C-terminal sequence called FXPRLamide where $X = V$, T, S or G (Abernathy *et al.*, 1995).

Cuticular melanization in lepidopteran insects appear to be regulated by PBAN or hormone(s) belonging to the PBAN family (Matsumoto *et al.*, 1990). This finding was later confirmed in *Pseudaletia separata* in which melanization is induced by Pss-pheromonotropin (Matsumoto *et al.*, 1992). Furthermore, the same neuropeptide was shown to induce embryonic diapause in *Bombyx mori* (Matsumoto *et al.*, 1992). In this study, PBAN secreting cells were localized in larval nervous system and alimentary canal of the castor semilooper, *Achaea janata*. In the light of the literature probable functions of this neuropeptide in addition to pheromone biosynthesis are discussed.

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Brain, suboesophageal ganglion, ventral nerve cord ganglia and different regions of the gut were dissected out and fixed in aquous Bouin's fixative and paraffin blocks were prepared. Immunocytochemical studies were conducted according to the method of Schoonveld and Veenstra (1988). Microtome sections (6 μm) of tissues were deparaffinated in xylene, rehydrated, rinsed in PBS and immersed the slides in 0.05% H_2O_2 in PBS for blocking endogenous peroxidase activity. Sections were treated with 10% normal goat serum for 10 minutes. Incubated overnight at 4°C in anti-PBAN raised in our laboratory against synthetic Hez-PBAN (Peninzula Lab, Belmont, CA) in rabbit. Slides were again rinsed and treated with blocking serum followed by secondary antibody (peroxidase labelled goat antirabbit Ig from Bangalore Genei) for 1 hr at room temperature. Rinsed again and visualized with a mixture of PBS, H_2O_2 and 3,3′ diamino benzedene tetrahydrochloride (DAB-HCl). Dehydrated in alcohol series, cleared in xylene and mounted in DPX.

Wholemount immunostaining was also carried out to confirm the presence of immunoreactivity according to the method of Ying *et al.* (1998).

Control slides were prepared by:

(i) Omission of primary antibody to check unspecific binding of secondary antibody and other compounds applied in the procedure.

(ii) Replacing specific antisera with pre-immune serum.

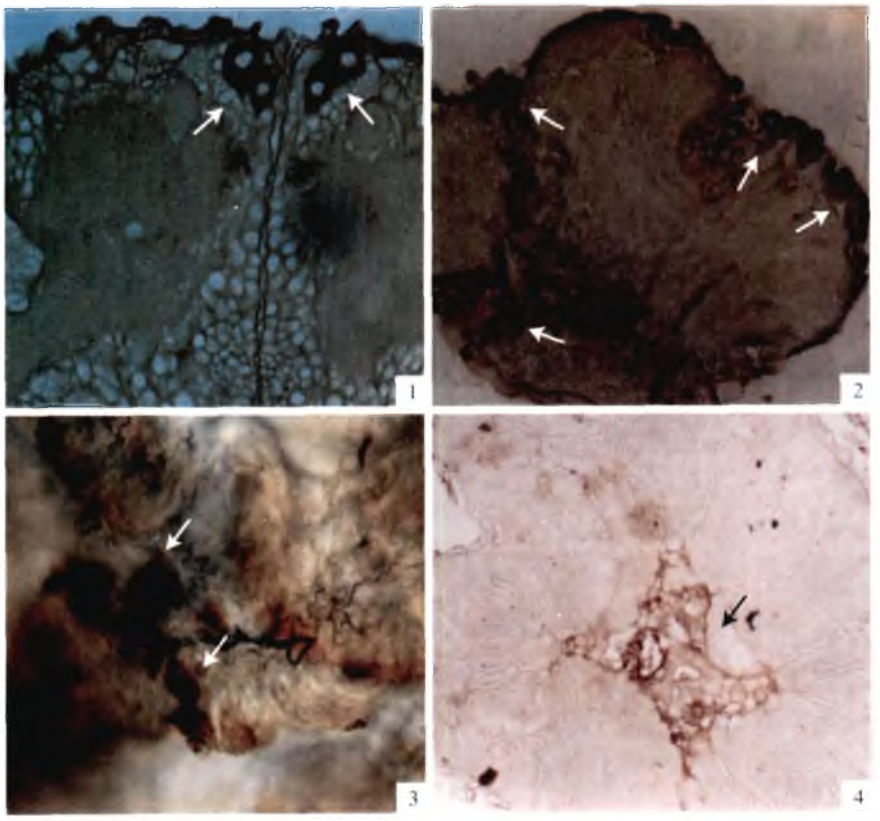
(iii) Pre-adsorption of antiserum overnight with 0.001/mg/ml Hez-PBAN

In larva, PBAN-like immunoreactivity was found thoughout the central nervous system. In the brain 3 pairs of immunoreactive somata could be located in each half of the protocerebrum (Fig. 1). In the suboesophageal ganglion (SOG), PLI was localized mainly in three cell groups located along the ventral midline of the ganglion (Fig. 2). In the most anterior cluster there were 4 somata, immediately posterior to this, a group of cells containing 8–10 numbers. Near the posterior end of the SOG lied 2 distinct immunopositive somata. In the corpora cardiaca immunoreactive axons (Fig. 2) could be located. The thoracis and abdominal ganglia also showed immunopositive reaction to anti-PBAN.

Some of the epithelial endocrine cells of the hindgut region showed immunopositive reaction in both sections and wholemount preparations (Fig. 3). High amount of immunoreactivity in the form of a network was detected in the midgut/hindgut boundary (Fig. 4).

No immunostaining was detected when pre-immune serum was used and PBAN-like immunostaining was completely abolished when the antiserum was pre-adsorbed overnight with Hez-PBAN.

In larvae of *Achaea janata*, PBAN-like immunoreactivity is located in both brain and VNC ganglia. Previous studies have shown the presence of PLI in larval head extracts of *Heliothis peltigera* (Gazit *et al.*, 1992). The presence of PBAN in early developmental stages, where sex pheromone biosynthesis does not take place, supported the findings of Matsumoto *et al.* (1988, 1990) that PBAN is involved in other functions in addition to sex pheromone biosynthesis.



FIGURES 1–4: 1: Sagittal section of the larval brain showing PBAN-immunoreactive cells in the protocerebrum (arrow marks) (Scale bar: 40 μm). 2: Three groups of immunoreactive cells in the SOG (arrows) and axons in the CC (arrowhead) (Scale bar: 20 μm). 3: Immuno wholemount of hindgut showing PBAN-like immunoreactivity. (Scale bar: 40 μm). 4: Immunoreactivity exhibited by midgut/hindgut boundary in the form of a network. (Scale bar: 40 μm).

Ogura and Saito (1972) and Ogura (1975) have shown that brain–CC–CA complex and the SOG are responsible for the melanization of the cuticle in *L. separata*. This neurohormone was characterized and primary structure was revealed by Matsumoto *et al.* (1990) and found to be an amidated peptide, consisting of 33 aminoacids. Comparison of its aminoacid sequence with that of other insect neuropeptides revealed full homology with PBAN isolated from the same moth (Kitamura *et al.*, 1989) and 80% homology with PBAN isolated from *H. zea* (Raina *et al.*, 1989). In addition, natural and synthetic Bom-PBAN induced cuticular melanization in the larval stages and sex pheromone production in adults (Matsumoto *et al.*, 1990), indicating that the same neurohormone may control different functions at different developmental stages.

PBAN-like immunoreactivity could also be detected in the midgut/hindgut boundary and in the hindgut regions of *A. janata*. In *Dysdercus cingulatus* also some of

the midgut epithelial cells reacted positively to the PBAN-antiserum (Ajitha and Muraleedharan, 2001). Gastric nerves link the stomodeal nervous system with proctodeal innervation at the midgut/hindgut boundary forming a network of the fine fibers. Here in *A. janata*, this boundary region contained a network of immunoreactive fibers. Allatotropin which stimulate the biosynthesis of juvenile hormone, earlier detected only in brain was recently demonstrated to inhibit midgut transport in *Manduca sexta* (Yeoll *et al.*, 1998). In *H. armigera*, allatostatins viz. Helicostatins were expressed in neurons of the central nervous system and endocrine cells of the midgut indicating that they are true brain-gut peptides (Davey *et al.*, 1999). *In situ* hybridization an immunochemical methods showed that in Drosophila neuropeptide F (NPF) was expressed both in the brain and midgut of fly larvae and adults (Brown *et al.*, 1999).

In the present studies, presence of PBAN-positive axon projecting into the VNC and immunoreactivity in the midgut/hindgut boundary show that PBAN is likely to be the transported axonally to release sites outside the head. This distribution may be compared with that of eclosion hormone of *Manduca sexta* which is produced in the brain neurosecretory cells. Axons of the cells project into the ventral nerve cord for central release of EH, but they also contain branches that exit peripherally via the proctodeal nerve to terminate at release sites on the gut (Truman and Copenhaver, 1989).

The distribution of PBAN-like immunoreactivity observed in both CNS and alimentary canal of larvae suggests a multiplicity of functions other than pheromone production. Bom-PBAN gene might code for four other neuropeptides which regulate pheromone production, induction of embryonic diapause, melanization of the integument and also stimulation of muscle contraction in insect gut (Kawano *et al.*, 1992). These peptides share with PBAN the C-terminal pentapeptide sequence. There is also evidence that some gut endocrine cell products enter the body circulation and exert hormonal effects on distal targets (Sehnal and Zitnan, 1996). Due to the various routes of action thus a single hormone can exert a variety of effects in insects.

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Influence of Worker Brood Survival Rate on the Performance of *Apis mellifera* L. (Hymenoptera: Apidae) Colonies During Different Seasons

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ABSTRACT: In the present studies, the colonies of *Apis mellifera* having high, medium and low brood survival showed significant variations in the bee population only during spring and not during summer and autumn. The low brood survival colonies also had significantly less brood in the spring season as compared to the normal colonies. During spring, the empty cells in the brood comb were 20.62 per cent in the colonies of low brood survival group as compared to the 9.47 per cent in high brood survival colonies. Maximum empty cells were found in all the colonies during autumn season.

KEYWORDS: Worker brood survival, *Apis mellifera*, colony performance.

Honeybees are contributing beneficially to agriculture to the extent that they support world-wide industries. The bees have been domesticated as well as bred by humans because of their valuable products. The economic value of honeybees has attracted the attention of several geneticists and breeders to carry out the breeding programmes of bees.

Maximum productivity is obtained from the colonies with high survival rate of brood which depends on the type of mating taking place and may range between 50–100 per cent. Only the brood heterozygous at sex locus survives, developing into workers. The colonies with low worker brood survival rate will be weak to yield a good honey crop. In our preliminary work (Chaudhary *et al*, 1998) it has been observed that the worker brood survival in *A. mellifera* colonies varied between 74.1 and 96.6 per cent. The studies have also revealed that colonies of high worker brood survival group had better hygienic behavior, recruited more bees and had more food stores than the colonies of lower brood survival group (Chaudhary and Gupta, 2000). In the present study the performance of the colonies belonging to different worker brood survival groups was recorded during different seasons.

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The worker brood survival rate of 45 colonies of *A. mellifera* was determined in the apiary of Department of Entomology and Apiculture, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan as described earlier (Chaudhary *et al*, 1998). On the basis of brood survival, the colonies were broadly grouped in to three categories.

(i) High brood survival rate (90–99% brood survival).

(ii) Medium brood survival rate (80–89%).

(iii) Low brood survival rate (70–79%).

For further studies three bee colonies were selected randomly from each of the above mentioned groups and detailed observations were recorded on these selected colonies. The data were recorded on bee population, brood area and food stores during summer, autumn and spring at 21 day interval. To estimate the number of bees per frame photographs of the combs covered with bees were taken. The number of bees on each frame was counted from the photographs. The population of the colony was calculated by multiplying the number of bees on a single frame (average of 6 observations) with the number of the frames covered with bees. Brood area and pollen stores were measured using a grid (having each square of 6.25 sq. cm) and the results were expressed in terms of sq. cm. The honey stores of the bee colonies were estimated by using grid used for measuring brood. The number of squares counted covering the honey area on each surface of the comb was multiplied by a factor of 7.8 (calculated on the assumption that each side of Langstroth frame scaled with honey has one kg of honey) and the results were expressed in kilograms.

To determine the proportion of comb used by the bees belonging to each group of brood survival, the brood area, food stores and empty comb area of the experimental colonies were measured at an interval of 21 days with the help of grid. The data were statistically analysed using complete randomized design after undertaking the necessary transformations where ever needed (Gomez and Gomez, 1986).

The influence of brood survival on brood and bee population has been reported to vary to a great extent during different seasons (Woyke, 1980, 1981). In the present studies during summer and autumn there were no significant differences in the colony population among the colonies belonging to different brood survival groups (Table 1). However, during early spring the worker population in the medium and low brood survival groups was 63 and 56.8 per cent of the colonies belonging to the high brood survival group.

The colonies of medium and low brood survival groups had on an average only 29.9 and 21 per cent of the brood area found in colonies of high brood survival group during early spring. However, during summer and autumn colonies of the low brood survival group had 42.44 and 50.45 per cent of the brood found in colonies of high brood survival group. In colonies of medium and high brood survival groups, differences in the brood area were non-significant during these seasons. The food stores which included both pollen and nectar, were significantly more in colonies belonging to high brood survival group as compared to those of medium and low brood survival groups.

TABLE 1. Seasonal variations in the performance of bee colonies having different brood survival rate

Season	Colony Parameters	Colonies of different brood survival rates			$CD_{0.05}$
		High (G1)	Medium (G2)	Low (G3)	
Summer	1. Woker population	13482.4	13127.6	13482.4	NS
	2. Brood area (cm^2)	2165.35	1424.6	918.89	778.89
	3. Honey stores (kg)	1.35	0.79	0.52	0.29
	4. Pollen stores (cm^2)	229.9	198.6	149.3	35.5
Autumn	1. Woker population	7894.3	7628.2	7894.3	NS
	2. Brood area (cm^2)	1249.96	953.44	630.57	345.45
	3. Honey stores (kg)	0.78	0.48	0.35	0.28
	4. Pollen stores (cm^2)	196.0	167.2	133.3	30.15
Early spring	1. Woker population	5388.53	3392.78	3060.15	1080.0
	2. Brood area (cm^2)	1315.63	393.63	276.25	G1 × G2 = 198.84 G2 × G3 = 340.06 G1 × G3 = 340.06
	3. Honey stores (kg)	0.44	0.34	0.20	G1 × G2 = 0.14 G2 × G3 = 0.19 G1 × G3 = 0.19
	4. Pollen stores (cm^2)	278.56	138.5	149.67	G1 × G2 = 67.03 G2 × G3 = 86.89 G1 × G3 = 86.89

The studies on the brood comb use in the colonies of the three groups revealed distinct differences which were also influenced with the season of the year. In spring, colonies having high, medium and low brood survival had, respectively, 9.47, 11.92 and 20.62 per cent of the brood cells empty. In summer, 12.73, 16.86 and 23.42 per cent cells were, respectively, empty. However, during autumn, the empty cells were maximum in colonies of all the groups, the respective values being 21.26, 25.25 and 29.24 per cent. Empty cells in the colonies of low brood survival group were significantly more than in the colonies of high brood survival group duing all the periods of observations. Woyke (1980) while comparing the worker population and brood comb area during summer found that in colonies having 100, 75 and 50 per cent brood survival, the colonies of low brood survival group had 35 per cent of worker population as compared to normal summer population. During spring and autumn, the worker population in these colonies was 79 and 65 per cent of the normal population (of colonies having 100 per cent brood survival). In the present studies we have found significant variations in the bee population only during spring and not during summer and autumn among the colonies of the three groups of honey been colonies. Interestingly, the colonies of low brood survival group also had significantly less brood in spring than those of normal colonies. The empty cells in the brood comb during

spring were 20.62 per cent in the colonies of low brood survival group as compared to 9.47 per cent in high brood survival colonies. The maximum empty cells in all the colonies were during autumn whereas Woyke (1984) found maximum empty cells in summer where a large comb area was used for brood rearing. The variations might be attributed to the prevailing floral conditions during different seasons as well as varying strength of bee colonies in these studies. During autumn, in the present studies through brood area was small yet queen was not effective at replacement egg laying. Thus it seems possibly floral conditions which influence colony strength also play an important role in the brood comb use by the queen. The worker population in early spring in the present studies ranged between 3060 to 5388 bees/colony as compared to 13,800 bees/colony during spring in the normal colonies in case of studies made by Woyke (1980, 1981).

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Natural Enemies of Ber Hairy Caterpillar *Thiacidas postica* Walker (Lepidoptera: Noctuidae) in Karnataka

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ABSTRACT: The hairy caterpillar *Thiacidas postica* Walker (Lepidoptera: Noctuidae) on ber was found attacked by four parasitoids, namely, *Exorista* sp., *Chaetexorista* sp., *Charops obtusus* Morl. and *Apanteles creatonoti* Vireck and one nuclear polyhedrosis virus. The total mortality due to these bioagents ranged from 14.9 to 26.8% during August–December, 1999. *Exorista* sp and *Chaetexorista* sp appeared to be new records on *T. postica*.

KEYWORDS: Ber, *Thiacidas postica*, parasitoids, nuclear polyhedrosis virus.

Thiacidas postica Walker (Lepidoptera: Noctuidae) is a sporadic pest of ber *Zizyphus mauritiana* L.). The first three instars of the ber hairy caterpillar feed by scraping the chlorophyll content and skeletonising the leaves. Later instars consume entire leaves leaving behind only the midrib and petiole (Mehra and Sah, 1976; Devaiah *et al*, 1983; Pradeep, 1994). Incidence of the hairy caterpillar was observed on ber (cv. Umraon) from August to December 1999 at the Indian Institute of Horticultural Research Farm, Hessaraghatta, Bangalore. The larvae collected from five ber trees were fed with fresh leaves daily in the laboratory until pupation. The number of adult moths and parasitoids emerged was recorded to work out the per cent parasitism. The dead caterpillars were examined for the presence of pathogens. The indentity of the moth was determined at the University of Agricultural Sciences, Bangalore, and that of the parasitoids at the Project Directorate of Biological Control, Bangalore.

Field collections of *T. postica* had yielded *Exorista xanthaspis* Wied, *Chaetexorista* sp. (Diptera: Tachinidae), *Charops obtusus* Morl. (Hymenoptera: Ichneumonidae) and *Apanteles creatonoti* Vireck (Hymenoptera: Braconidae) and a nuclear polyhedrosis virus (NPV). *Charops obtusus* and *A. creatonoti* parasitised early instars while the tachinids were larval-pupal parasitoids emerging from the pupae of *T. postica*. The per cent parasitism during the observation period chiefly by *Exorista* sp. and *C. obtusus*,

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TABLE 1. Natural parasitism on ber hairy caterpillar *Thiacidas postica* at Bangalore in 1999.

Month	% parasitism*		% NPV	% total
	Exorista sp.	*Charops obtusus*	infection*	natural mortality*
August	11.42	5.71	2.85	14.93
September	5.95	11.90	3.57	21.42
October	11.94	13.41	1.49	26.84
November	11.86	7.60	3.38	22.84
December	8.10	8.10	1.35	17.55

* Number of larvae collected = 59–74 month^{-1}.

ranged from 16.2 to 26.6%, respectively. The NPV was found infecting the larva and the per cent natural infection in the field ranged from 1.4 to 3.6%. Thus, the total natural mortality of *T. postica* due to bioagents ranged from 14.9 to 26.8 per cent.

Mehra and Sah (1976) recorded the parasitoids *Apanteles taprobanae* cameron *C. obtusus*, *Goryphys* sp. and *Brachymeria* sp. on *T. postica*. According to Pradeep (1994), third and fourth instar larvae of *T. postica* were found attacked by the parasitoid *Dolichogenidea hyposidrae* Wilkinson during July–August and the parasitism ranged from 14.8 to 18.0%. Sathe (1988) earlier recorded *Apanteles creatonoti* on *T. postica*. Further, Pradeep (1994) also reported that *T. postica* found to be infected by NPV with 19.2% mortality under field conditions during September and October. The present record of *Exorista* sp. and *Chaetexorista* sp. appeared to be new on *T. postica*.

The total natural mortality caused by the five bioagents in the present investigation ranged from 14.9 to 26.8%, which was not adequate to suppress the population of *T. postica* effectively. Since it is very difficult to multiply the tachinid parasitoids in large numbers, the production and the use of NPV is suggested against *T. postica* to supplement the natural mortality by the local parasitoids.

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The Phenology and Predatory Behaviour of *Pardosa birmanica* Simon (Araneae: Lycosidae) on Insect Pests of Cotton

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ABSTRACT: *Pardosa birmanica* Simon was found one of the dominant spiders observed in the cotton agroecosystems of North Gujarat. The feeding capacity of this spider on different pests of cotton was evaluated in the laboratory. The rate of predation was found varying in different developmental stages and between the sexes. Subadult and adult females were found to be having a high feeding capacity on four insect pests of cotton.

KEYWORDS: *Pardosa birmanica, Aphis craccivora, Ambrasca biguttla, Heliothis armigera, Tricentrus bicolor*, feeding potential.

Cotton is an important cash crop cultivated almost all over the world. In India it is cultivated over 74 million hectares mainly in Maharashtra, Gujarat, Andhra Pradesh, Tamil Nadu, Punjab, Karnataka, Madhya Pradesh and Rajasthan. Chemical insecticides are one of the effective tools available for combating the pest problems. However, over-reliance on them has caused many problems such as environmental pollution, destruction of natural enemies, development of resistance and resurgence of pests. Biological methods of pest control offer great scope in this regard (Dhulia and Yadav, 1994). Spiders have attracted attention as predators of crop pests in several countries during the past few decades. They form one of the ubiquitous groups of predaceous organisms in the animal kingdom (Richert and Lockley, 1984).

The presence of arthropod predators in cotton fields and the efficiency of spiders as a potential biological control agents has been of great interest (Kagan, 1943; Whitcomb *et al.*, 1963; McDaniel and Sterling, 1979, 1982; Bishope and Blood, 1981; McDaniel *et al.*, 1981; Dean *et al.*, 1982). A quantitative estimation of population densities and seasonal abundance of the predatory spiders was carried out in cotton fields of North Gujarat during 1983–86 (Sebastian, 1988). *Pardosa birmanica* was one of the dominant spiders found in the cotton agroecosystems. The predatory potential of the spider was evaluated in the laboratory.

Spiders were collected randomly from twenty-five plants from cotton fields (5 hect. each) at Bayad, Modasa, Himatnagar, Sami, Vijapur and Surendranagar in North Gujarat. Collections were made every month throughout the cropping season from August to March during 1983–86 to study the seasonal abundance and phenology. Field collected and laboratory reared III and IV instar spiderlings, sub-adult and adult males and females of *P. birmanica* were taken to study the predatory potential. A definite number of pests were placed on twigs of cotton plants with 2–4 leaves, covered with bell jars. The cut end of the twigs was kept immersed in a beaker containing water. The test spiders were starved for 24 hours prior to the experiment and were placed individually on cotton twigs. The addition of prey was made at such a frequency that the prey density remained constant throughout the trial. Nymph stages of jassids, aphids and early instar larvae of, cotton bollworm and tur tree stem borer were subjected to predation. Preys consumed or killed by the spiders were counted at every 6 hrs. interval for 3 days for each trial.

P. birmanica was found abundant throughout the cropping season. The spider composition according to the method of prey capture was as with hunting spiders comprising 67.21% of the total collections. The hunting spiders group comprised of the families Lycosidae, Gnaphosidae, Clubionidae, Salticidae and Oxyopidae. At all the sites, lycosids were the dominant spiders. The relative composition of the hunting spider families was not significantly different among the six sites. *P. bimanica* was the most abundant species forming 17.42% of the total collections, 42.28% of the lycosids and 25.92% hunting spiders. All the dominant lycosid spiders including *P. birmanica* reached their peak in numbers during October. They were seen in minimum numbers during December and January and increased again in February and March.

The population density increased steadily from August and rose to its peak in October [Fig. 1(a)]. A drop in abundance was observed in November and still less during December–January. A slight increase in the population was noticed from February onwards. All the three forms like male, female and juvenile were observed in the month of August. Numerically a peak in male population was observed in October but completely absent in December. Again, the males were reappeared only in February. Females appeared from August and reached to their peak in October. There was a decrease in the population of the female from November to January again with further increase in February. Males were found throughout the cropping season except during the winter months. Juveniles were found throughout the cropping season with their peak in numbers from September to December [Fig. 1(b)]. The phenology of this species was found similar to other dominant hunting spiders of the family Lycosidae in cotton fields. Numerically *P. birmanica* was found to be I in rank 17.42% and the family Lycocidae also ranked first with 41.19% of the total collections at all sites. This fluctuation in the population supports the hypothesis of Pianka (1974) that the more productive habitats with the availability of surplus insect prey, reduced microclimatic changes and increased structural retreats supports the abundance as the cotton plants starts budding and flowering during the post monsoon months. *P. birmanica* is a hunting spider and found always on the ground stratum. It is evident from the studies

TABLE 1. Feeding potential of different stages of *P. birmanica* on insect pests of cotton (Average of 10 replica)

	Average number of pests consumed in 24 hrs.										
Pests	III Instar	IV Instar	Sub-adult male	Sub-adult female	Adult male	Adult female	Rank max feeding	Mean	SD	CV%	Rank feeding consistency
Aphis craccivora	17.2	21.2	31	38.6	21.2	48.2	I	29.57	10.97	37.09	I
Amrasca biguttula	8.3	12.6	21.4	28.3	31.2	42.6	II	24.06	11.56	48.05	II
Heliothis armigera (3–4mm long)	8.2	12.1	16.3	21.3	14.1	28.4	III	16.73	6.57	39.27	III
Tricentrus bicolor (1-2mm long)	8.5	11.6	15.8	17.5	15.2	19.5	IV	14.68	3.67	25	IV

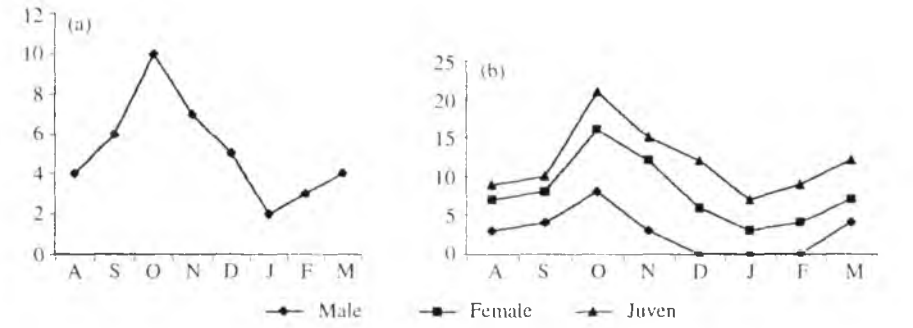

FIGURE 1. (a) Seasonal abundance of *P. birmanica* in cotton fields, (b) Phenology of *P. birmanica* in cotton fields.

that this spider occupying the fields reproduced there and their number increased considerably in the fields during the post monsoon season. This spider was found most active during the months September to November, which was wet but warmer than the preceding and following months and was supported by greater prey abundance.

P. birmanica was found to pounced over the prey and hold them with the stout and toothed chelicerae. It consumes the prey by sucking the body fluid through the insertion made at the joints of the legs or on carapace. The rate of predation was found varying among different developmental stages and between sexes (Table 1). The rate of predation ranged from 17.2 to 48.2 on aphids, 8.3 to 42.6 on jassids, 8.2 to 28.4 on bollworm and 8.5 to 19.5 on tur tree borer per day. The rank of pests as per the feeding preferences and consistency in feeding by *P. birmanica* was found to be the highest of *Aphis craccivora* and the lowest of *Tricentrus bicolor* (Table 1). Adult and subadult females were found to be having a higher rate of feeding potential. The III and IV instar spiderlings also showed a high feeding potential. The sub-adult male was to be having high feeding potential compared to adult male. When equal number of all the four candid pest were subjected to predation the order of prey preference was found aphids > jassids > bollworms > tur tree borer.

It is difficult to relate the rate of prey consumption in the laboratory to that in the field. Individual feeding does not mean too much (Whitcomb *et al.*, 1963) but each result does indicate that this species could and did feed on the particular prey. Spider's preference to their prey is a matter of discussion. Bilsing (1920) stated that there is no evidence that any species of spider has a particular to the prey. Turnbull (1960) pointed out that spiders will tolerate a wide range of insect pests and preferred pest will vary from time to time and from place to place depending on the particular time and place.

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Some Observations on the Fig Moth, *Cadra cautella* (wlk.) Infesting Onions in Storage

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ABSTRACT: The fig moth *Cadra cautella* (W.) infestation was studied in storage onion bulbs. *C. cautella* infestation in storage increased with storage length, probably with decrease in relative contents of moisture, pyruvic acid and total sulphur. Removing the dry outer peels of bulbs reduced the damage caused by *C. cautella* considerably. Biology of the insect also was studied.

KEYWORDS: *Cadra cautella*, onion, pyruvic acid, sulphur.

The fig moth, *Cadra cautella* (Lepidoptera: Phycitidae) infests dried vegetables, fruits and other food products in storage (Arnold, 1969). Dehydrated onions (*Allium cepa* var. *aggregatum*) packed even in polythene bag suffer damage (Mohan Rao *et al.*, 1972). The pest develops rapidly when onions get dried (Lal and Varma, 1974). After hatching, the caterpillars enter the bulbs either through the outer leaf or cut end of the shoot, eating irregularly causing webbing of leaves together with the excreta and finally making the entire bulb loose (Lal and Varma, 1974). The observations made on the biology and damage in relation to the biochemical profile of onions at various storage lengths are presented here.

The experiments were conducted in the laboratory during February, 1997 at Agricultural College and Research Institute, Killikulam where the ambient temperature was 29.0 ± 2 °C and the relative humidity was 75.0 ± 1.0%. Eggs were easily collected by placing adult moths in polythene bag, males and females mixed. The next day eggs were transferred to open petriplates containing onion bulbs placed inside the culture cages. Observations on egg, larval and pupal periods and adult longevity were recorded daily in the morning. The bulbs were changed every week after carefully transferring the larvae to another petriplate till adult emergence.

The level of damage to onions after various storage periods was assessed by exposing onion samples in petriplates to *C. cautella*. Ten eggs were added to each petriplate. Hundred bulbs were maintained for each storage duration. The percentage of damage was assessed after 25 days of egg inoculation. In another experiment,

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TABLE 1. Biology of *C. cautella* on onion bulbs in storage

Stage	Period in days ± SEM	Range in days
Egg	4.69 ± 0.17	4–5
Larva	30.70 ± 1.69	25–42
Pupa	7.44 ± 0.47	5–11
Total	42.83 ± 2.33	34–68
Adult longevity	5.02 ± 0.35	3–7

seven months old dehydrated onions bulbs were subjected to *C. cautella* attack with or without outer loose peels. Hundred bulbs were tested for each category in ten petriplates to which *C. cautella* eggs were added at the rate of 10 eggs per plate. The infestation was examined after 25 days. Pyruvic acid, sulphur content and moisture content of bulbs were also estimated from the bulb samples drawn at various storage length (Chesnin and Yien, 1951; Hart and Fisher, 1971).

Greyish female moths laid whitish spherical eggs singly in between the loose outer peels of onion bulbs. Eggs hatched in 4–5 days, the average being 4.69 days (Table 1). Newly hatched larvae were pinkish white in colour. The larval period lasted 25–42 days averaging 30.7 days. Dark brown mature larvae bored into the inner core of bulbs where they pupated after spinning a silken cocoon. The pupal period averaged 7.44 days. Adults lived for 3–7 days. The total life cycle occupied 34–68 days, average being 42.83 days. In other studies earlier the total life cycle has lasted 35–42 days (Lal and Varma, 1974) and 47–56 days (Mital, 1967). This variation in biology could be due to variations in climatic conditions from place to place.

The pest inflicted no damage to freshly harvested bulbs (Table 2). On the other hand, no bulbs were spared from damage after 7–9 months of storage. It was evident that larvae of *C. cautella* did not damage freshly harvested bulbs and that the damage level increased with increase in length of storage period. Only 15 percent of the bulbs were damaged after one month storage. The infestation increased five times after 7–9 months. This may be due to the reduction in the level of moisture, pyruvic acid and total sulphur (Table 2), the contents of which showed negative relationships with the pest attack. Freshly harvested onion bulbs had no damage most probably due to higher levels of moisture in the bulbs. In addition, the profile of pyruvic acid and total sulphur would probably be responsible as deterrent factors to *C. cautella*. Earlier studies have also indicated that dried bulbs are more favourable to the larval development (Mohan Rao *et al.*, 1972). As pyruvic acid and sulphur compounds are characteristic in imparting pungency to onions (Schwimmer and Weston, 1961; Schwimmer, 1971), freshly harvested bulbs are more pungent than dehydrated bulbs.

Experimentally, removing the loose outer peels of onion bulbs reduced the damage potential of *C. cautella* larvae (Table 3). Caterpillars damaged 96.7 percent of bulbs when none of the outer peels was removed. After peeling off a single outermost leaf from the bulbs, the caterpillars damaged only 70.0 percent of bulbs. Removal of the

TABLE 2. Relative contents of moisture, pyruvic acid and total sulphur and damage to onion bulb caused by *C. cautella* after variable length of storage

Storage duration	% damage	Moisture content (%)	Pyruvic acid (mg/g)	Total sulphur (mg/g)
Zero storage	0.0	78.0	4.7	4.374
1 month	15.0 ± 5.81	70.0	5.2	4.852
5 months	73.3 ± 4.41	65.2	2.8	4.274
7 months	100	62.0	2.5	3.502
9 months	100	61.1	1.7	2.720

TABLE 3. Effect of removal of loose outer peels of onion bulbs on *C. cautella* damage

Bulb type	% damage
Unpeeled	96.7 ± 3.33
One peel off	70.0 ± 5.77
Two peel off	16.7 ± 3.33

Values represent percentage ± SEM.

two outer leaves reduced the level of damage to as low as 16.7 percent. It is probable that deleafed bulbs would not support *C. cautella* eggs in position so that the first instar larvae may have difficulty in gaining entry into the bulbs. Alternatively, it is possible that inner leaves which are less dehydrated than the outer ones may be less favourable to the early instar larvae to enter.

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Description of the External Morphology and Genitalia of Mango Stone Weevil *Sternochaetus mangiferae* Fab. (Curculionidae: Coleoptera)

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ABSTRACT: External morphological characters as well as structure of genitalia of adult male and females of mango stone weevil *Sternochaetus mangiferae* (Fab.) were described in detail. Adult sexual description was done based on size and shape of abdomen.

KEYWORDS: mango stone weevil (*Sternochaetus mangiferae*), external morphology, genitalia.

INTRODUCTION

Mango stone weevil *Sternochaetus mangiferae* Fab. is one of the important pests of mango under sub-tropical climatic conditions. Though the bio-ecology of this pest was studied by Seshagiri Rao *et al.* (1971) and Shukla and Tandon (1985), the descriptions of the adult male and female stone weevils were scanty. Hence an attempt has been made to study the external characters of both male and female of stone weevil including the morphology of genitalia. The works of Mann and Singh (1979), Pajni and Sidhu (1982) and Gilber and Edward (1951) on different coleopterans including Curculionid weevils were taken as guide lines in documenting the information on *S. mangiferae*.

DESCRIPTION

Body is dark brownish black, oval, compact and convex in shape with white markings on the prothorax and black hue on the elytra. Body is densely clothed with arenacious, recumbent, fan shaped short scales.

Head is strong, rostrum subcylindrical, slightly curved and dark brown in colour. When the weevil is at rest, rostrum is inserted into groove located in the prosternum in between the coxae of the prolegs and middle legs. Vertex raised continued with

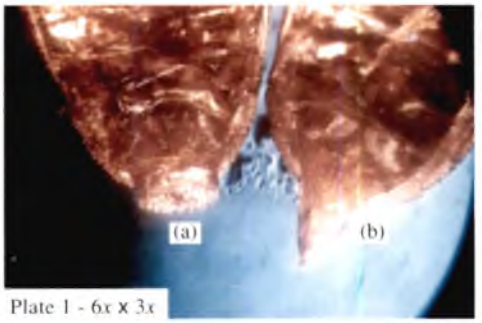

Plate 1 - 6x x 3x

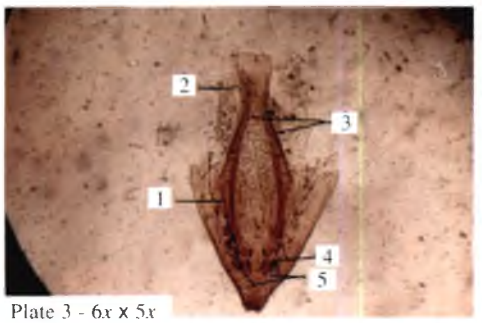

Plate 3 - 6x x 5x

1. Coxite
2. Spiculum ventrale
3. Lateral arms
4. Styli
5. Setae

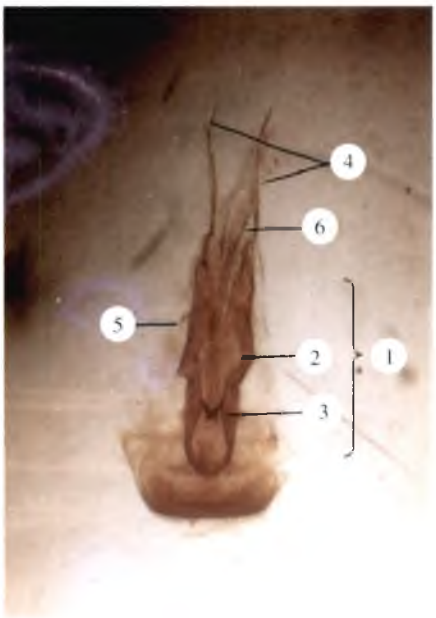

Plate 2 - 6x x 5x

1. Aedeagus
2. Phallotreme
3. Orificial plates
4. Aedegal apodeme
5. Phallobasic ring
6. Ejaculatory duct

PLATES 1–3: Genitalia of stone weevil. 1: Ventral side distal parts of a. Male, b. Female weevil. 2: Male genitalia 3: Female genitalia

rostrum, eyes black, antennae geniculate with nine segments arise from the apex of the rostrum. First segment of antennae is very long, inserts in the antennal groove on lateral sides of rostrum. The last ninth segment is club shaped densely covered with thick glandular hairs. Other segments have sparsely long hairs.

Pronotum broad at base, triangular in shape with white vertical line on the centre.

Elytra ferruginous, broader than pronotum, completely covers abdomen. Hind wings are membranous, longer than elytra and remain folded under elytra.

Legs with three tarsi, third tarsi brachirhinous type, densely covered with smooth glandular hairs.

Male and female weevils have similar abdominal segments on ventral side. There was no much difference in shape and size of the adult mango stone weevils. In females last segment of the abdomen was narrow at base with raised constriction on ventral side. Whereas in males, last segment of the abdomen was broader than females with furrow like markings on ventral side (Plate. 1).

Male genitalia

Aedeagus straight, bilobed, broad at base and strongly sclerotised. Exophalic valve present at the centre of basal region of aedeagus. Phallotreme is parallelogram in shape, located at the centre of aedeagus, with orifical plates at base. A pair of narrow, gently curved, thin and slender aedeagal apodemes, shorter than aedeagus were raised from the apex of the aedeagus. Phallobasic ring surrounds the aedeagus on its sub apex. Below the junction of aedegal apodeme, short, narrow needle like phallobasic apodeme was located. The ejaculatory ducts were thin, long and narrow, raised from the base of the aedeagus, endophallus did not surpass the aedegal apodemes (Plate 2).

Female genitalia

Female genitalia with highly sclerotised ovipositor, long tubular coxites with forceps like spiculum ventral. Lateral arms inserted into coxae. Medium arm short and broad at its apex. Styli cylindrical, straight and attached at the base of the stylus. Tuft of hair like setae at the base of the stylus were identified (Plate 3).

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Mating and Oviposition Cage for *Helicoverpa* during Winter Season

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ABSTRACT: The mating and fecundity of *Helicoverpa* spp. are greatly influenced by temperature and humidity. During winter, particularly in the northern part of India barring high altitude regions, the room temperature dips down to about 15 °C. The relative humidity also prevails between 20–50 percent. Such conditions are generally unfit for the insects and particularly *Helicoverpa* to breed. Therefore, the mating and oviposition cage for *Helicoverpa* for the winter season has been designed where ambient microclimate could be created. The oviposition cage is a cylindrical frame of galvanized iron wire enclosed in cotton cloth, which in turn is housed in a closed plastic container having little water in the base. A 100 Watt electric lamp is positioned at the top of the container to provide warmth. The height of the lamp is adjusted so as to maintain the inside temperature at 27 ± 2 °C. The water in the closed plastic container maintained 80 ± 5 percent relative humidity. Under these conditions, *Helicoverpa* mated and laid fertile eggs.

KEYWORDS: *Helicoverpa armigera*, oviposition cage, winter.

Helicoverpa spp. are the key pests causing colossal loss in cotton, pulses, vegetables, cereals and many other crops of economic importance. The genus has developed resistance against almost all known chemical pesticides. Nuclear polyhedrosis virus (NPV) of *Helicoverpa armigera* plays important role in the management of this pest. Since NPVs are produced *in vivo*, their mass production requires mass rearing of host insect. The female *H. armigera* lay more than one thousand eggs in a span of about 8–10 days. The mating and fecundity is greatly influenced by temperature and humidity. Modelers have assumed field fecundities ranging from 500 to 3000 eggs per female (Knipling and Stadelbacher, 1983; Hartstack *et al.*, 1976; Stinner *et al.*, 1974), modified by host plant and temperature.

During summer, high temperature and low humidity are detrimental for laboratory rearing. Kumar and Ballal (1990) developed an improved oviposition cage, which helps in maintaining the ambient conditions in summer, suitable for mating and

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oviposition. The other oviposition chamber in use is the 3.79 litre paper carton (Burton, 1969; Raulston and Lingren, 1972). The limitation of these cages is that these cannot be used for the winter season. During winter, particularly in the northern part of India barring high altitude regions, the room temperature dips down to about 15 °C. The relative humidity also ranges between 20–50 percent. Such conditions again are unfit for this insect to breed. Maintaining ambient conditions (temperature 27 ± 2 °C and relative humidity >75 %) in the laboratory round the clock in winter is quite expensive and difficult.

For winter season, the oviposition cage earlier developed by Kumar and Ballal (1990) has now been modified and used in different way so as to create microclimate suitable for *Helicoverpa* mating and ovipositing.

The oviposition cage is made of cotton cloth supported on a cylindrical frame of galvanized iron wire as described by Kumar and Ballal (1990). This cage is housed in an opaque plastic container. Nearly 25 pairs of pupae, ready to emerge were kept in the folds of tissue paper in a petri dish over the plate. A 40 ml plastic cup containing 20% honey solution was kept on the disc as food for the emerging moths.

A plastic container sufficiently large to house the cage was used to create desired microclimate. Inside the container, the cage was kept over a 5 cm raised platform. Approximately 100 ml water was kept in the container, which remained below the platform. A 100 W electric lamp was positioned at the top of the container to provide it warmth [Fig. 1(a) and (b)]. The height of the lamp was adjusted so as to maintain the temperature in the container at 27 ± 2 °C. Three such cages were kept for study. To compare the effect of housing of the cage in the container, three more cages were kept without container in another room. The temperature and the relative humidity of this room were recorded. On 3rd day of emergence of moths, they were transferred to other cages. The device used for collection of *Corcyra* moths (Kumar and Jalali, 1993) was used here for transferring of *Helicoverpa* moths. The moth were transferred to new cages on alternate day till 13th day of emergence of moths. Every time with the change of cage, 20% fresh honey solution was provided to the moths. In the cages without container, no egg laying was noticed, hence the moths were allowed to remain in the same cage. However, the feeding was changed on alternate days. The eggs laid on the inside surface of the cages were recovered by dipping the clothes in 0.01% Sodium hypochlorite solution and then rinsing them in tap water. The eggs were recovered on alternate day from each cage and counted by the method described by Kumar and Ballal (1990). Based on the egg count on alternate day the age-specific fecundity was determined.

The egg laying was found only in the cages which were kept inside the container. There were no egg laying in the cages (without container) which were kept in room temperature and relative humidity. The preoviposition period was observed to be 2–3 days. The egg laying started on 3rd day after emergence and continued till 13th day. After the 13th day, the egg laying was negligible, however, the moth remained alive for several days. It is, therefore, desirable to discard the moths after collecting eggs till 13th day. The average fecundity per female was observed to be 1017. The percentage

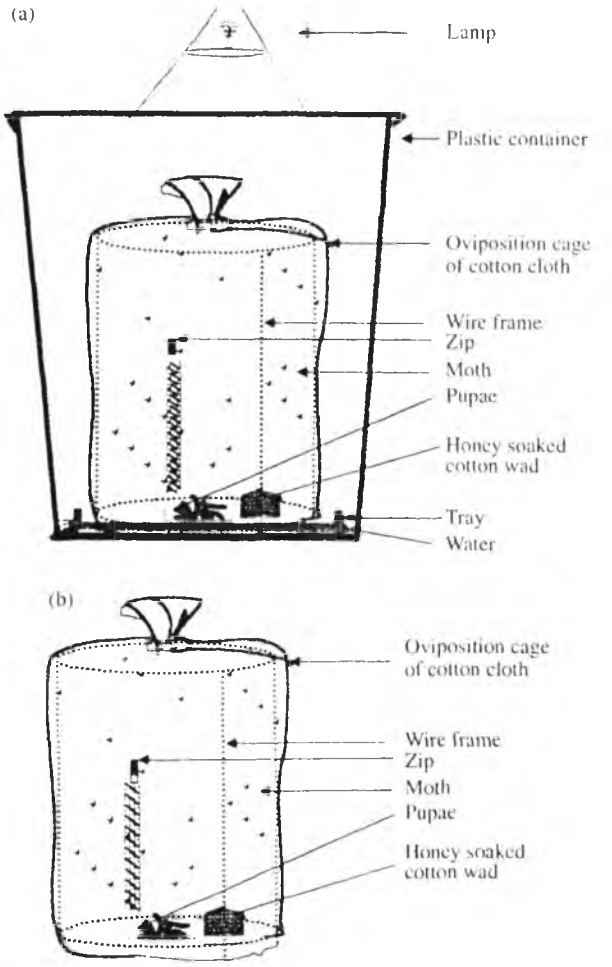

PLATE 1. (a) *Helicoverpa* oviposition cage for winter. (b) Inside of the cage.

egg laying on 3rd, 5th, 7th, 9th, 11th and 13th day was observed 1.43, 19.2, 39.9, 24.8, 11.1 and 3.7 respectively (Table 1). Upto 3000 eggs have been recorded from single *H. armigera* (Reed, 1965) and *H. zea* (Quaintance and Brues, 1905) females, but most estimates of fecundity, over the reproductive life time of about 8–10, range between 1000–1500 eggs per female (Fye and McAda, 1972). Fecundity is influenced by temperature, humidity and larval and adult nutrition (Adjei-Maafo and Wilson, 1983; Isely, 1935; Nadgauda and Pitre, 1983; Willers *et al.*, 1987). In the present study, the influence of host plant was not there which might further induce oviposition. Yet in the laboratory good oviposition was obtained by the system explained. At room

TABLE 1. Age specific fecundity of *H. armigera* (25 females in each cage)

Day after emergence	3rd day	5th day	7th day	9th day	11th day	13th day	Fecundity of 25 females	Fecundity per female
Cage 1	125	867	8750	7575	3917	1250	22484	899
Cage 2	700	5041	9866	5475	2642	250	23974	959
Cage 3	267	8742	11792	5858	1884	1292	29835	1193
Grand Total	1092	14650	30408	18908	8443	2792	76293	
Mean	364 (1.4)	4883 (19.2)	10136 (39.9)	6303 (24.8)	2814 (11.1)	931 (3.7)	25431	1017

Figures in parantheses are age-specific fecundity in percentage

temperature and relative humidity 16 ± 02 °C and 25–50 percent respectively no egg laying was observed.

It is evident that rearing of *Helicoverpa* during winter season in north India is now possible by creating microclimate in the manner explained.

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Food Consumption and Energy Budget of a High Altitude Grasshopper *Xenocatantops karnyi* K. (Orthoptera: Acrididae)

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ABSTRACT: The energy budget of *Xenocatantops karnyi* K. a native of high altitude forest of western Himalayas was studied in the laboratory. Third instar nymphs were collected from the study site and fed with grasses to determine the food consumption, assimilation and tissue growth rates. All these parameters increased with development and female had significantly higher rates of consumption and tissue growth than males but assimilation was comparatively higher in males than females. Ecological efficiencies increased with the development in both the sexes, while the approximate digestibility decreased from third instar to adult.

KEYWORDS: Grasshopper, high altitude, energy budget, *Xenocatantops*.

The size of a population and the interactions between populations within an ecosystem may be expressed in term of biomass and energy content, as well as numbers. Biomass and energy are useful to ecologists in that they provide a common unit for the description of population of animals and plants of different sizes. The concept of energy is fundamental in the consideration of the functioning of ecosystem (Ivlev, 1939, 1945; Lindemann, 1942). Construction of an energy budget requires the initial development of a numerical budget. Normally it is necessary to calculate the standing crop and caloric value together with the independent measurement of either consumption and excretion rates or respiration. Kitazawa (1959), stated that the energy budget of individual organism is a basic requirement for better understanding of ecosystem. Qualitative food requirements offer few differences that distinguish one species from other, specially among the phytophagous insects, so quantitative analysis are essential to understand the ecological relationships among organisms.

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Present investigation deals with the food consumption, assimilation, growth and efficiencies of food utilization by the third, forth and fifth instar nymphs and adults of *Xenocatantops karnyi* K. fed on the leaves of *Cynodon dectylon* (L) Pers.

Third instar nymphs of the grasshoppers *Xenocatantops karnyi* K. were collected from the north–west part of Nanda Devi Biosphere Reserve India, in May, 2000. The Nanda Devi Biosphere Reserve is situated between 30°17′ to 30°40° north and 79°40′ to 80°05′ east. The insects were brought to the laboratory at Hardwar, they were maintained in 0.1 m^3 cages at a temperature of 32 ± 2 °C and ambient relative humidity of 72%. *Cynodon dectylon* (L) Pers. was collected regularly from the forest close to University campus and used as food for the grasshoppers. The grass was kept fresh by placing them in plastic tubes filled with water. The tubes were closed in order to prevent insect faeces from falling into them and get mixed with water. Energy parameters of first and second instars were not studied because of the difficulties of handling these small nymphs and accuracy in measuring their food consumption. The grasshoppers were restricted from food and water for 30 mins before the start of the experiment to allow the passage of residual faeces from the gut.

Before the start of experiment, each grasshopper weighed and individually kept in a 500 ml glass jar. At each developmental stage, ten grasshoppers of each sex were allowed to feed on portion of pre-weighed grass for 24 hrs. After this period, the remaining grass and faeces were collected and oven dried to a constant weight at 80 °C. A wet/dry mass ratio was determined for the grass and the amount ingested by each grasshopper was estimated. Dry weight equivalents of grasshoppers were also calculated by using oven dried 50 grasshoppers. Food consumption was calculated as the difference between the initial weight of the grass and weight of the grass left at the end of experiment after 24 hrs. Net assimilation was calculated by subtracting the dry weight of the faecal matter from the consumed grass, while the increase in body weight was taken as a measure of tissue growth. Ecological efficiencies were calculated using Waldbauer's (1968) expressions.

The parameters, initial biomass, consumption, egesta, assimilation and tissue growth show a marked increase as a function of developmental stage (Table 1). The biomass, consumption and egesta were comparatively higher in females than males. The biomass in males ranged from 23.65 (3rd instar) to 56.12 (adult) mg $insect^{-1}$ day^{-1} while in female it ranged between 28.35 (3rd instar) to 104.30 (adult) mg $insect^{-1}$ day^{-1}. The average rate of consumption in males ranged from 14.78 to 30.92 mg $insect^{-1}$ day^{-1} while in females it ranged from 15.84 to 40.55 mg $insect^{-1}$ day^{-1}. Male grasshoppers had a slightly higher assimilation rate than females and it ranged between 9.23 to 17.84 mg $insect^{-1}$ day^{-1} and 6.79 to 16.52 mg $insect^{-1}$ day^{-1} respectively. Females had higher tissue growth than males. In males it ranged from 1.58 to 3.98 mg $insect^{-1}$ day^{-1} while in females it ranged between 3.02 to 7.88 mg $insect^{-1}$ day^{-1}. Matsumoto (1971), White and Watson (1972) and Vats and Kaushal (1981) reported that the rate of consumption and egestion were higher in females than males.

TABLE 1. Initial biomass, consumption, egesta, assimilation and tissue growth in *Xenocatantops karnyi* K.

State	Initial biomass (mg insect^{-1})	Consumption (mg insect^{-1})	Egesta (mg insect^{-1})	Assimilation (mg insect^{-1})	Tissue growth (mg insect^{-1})
			Male		
3rd instar	23.65 ± 1.12	14.78 ± 1.12	5.55 ± 0.57	9.23 ± 0.81	1.58 ± 0.26
4th instar	32.23 ± 1.14	24.64 ± 1.22	11.02 ± 0.69	13.62 ± 0.86	2.72 ± 0.20
5th instar	52.43 ± 1.03	27.36 ± 2.20	11.27 ± 0.48	16.08 ± 2.05	3.42 ± 0.35
Adult	56.12 ± 0.33	30.92 ± 0.28	13.08 ± 0.25	17.84 ± 1.04	3.98 ± 0.28
			Female		
3rd instar	28.35 ± 1.07	15.84 ± 0.87	9.05 ± 0.87	6.79 ± 0.69	3.02 ± 0.40
4th instar	42.05 ± 1.84	29.02 ± 1.37	16.58 ± 0.85	12.44 ± 1.22	5.58 ± 0.34
5th instar	70.07 ± 2.07	37.84 ± 1.83	21.67 ± 0.98	15.87 ± 1.33	7.28 ± 0.58
Adult	104.30 ± 0.73	40.55 ± 1.22	24.03 ± 0.25	16.52 ± 1.52	7.88 ± 0.42

TABLE 2. Efficiency of food utilization in *Xenocatantops karnyi* K.

Stage	Apparent digestibility (AD)	Efficiency of conversion of digested food (ECD)	Efficiency of conversion index (ECI)
		Male	
3rd instar	62.44 ± 3.51	17.11 ± 4.25	10.69 ± 1.21
4th instar	55.27 ± 2.22	19.97 ± 2.45	11.03 ± 0.89
5th instar	58.77 ± 2.46	21.26 ± 2.14	12.50 ± 1.05
Adult	57.69 ± 1.93	22.30 ± 1.27	12.87 ± 1.08
		Female	
3rd instar	42.86 ± 4.14	44.47 ± 2.04	19.06 ± 2.00
4th instar	42.86 ± 2.24	44.85 ± 2.22	19.22 ± 1.07
5th instar	41.93 ± 1.74	45.87 ± 1.73	19.23 ± 1.50
Adult	34.83 ± 2.96	47.69 ± 4.20	19.43 ± 0.67

Ecological efficiency is defined as a ratio of any two parameters of energy flow in or between the trophic levels of natural community, in or between population or organisms (Odum, 1959). The efficiencies of food utilization are presented in Table 2. Apparent digestibility was higher (62.44 ± 3.51) in third nymphal instars of males and third and forth instars of females (42.86 ± 4.14). The decline in digestibility from the fifth instar to adult may have been due to the fact that younger stages were fed on soft and more succulent tender leaves, whereas the adults were fed on leaves, which were more fibrous. The males were more efficient in assimilating the consumed food. These findings are in line with earlier studies by Kaushal and Joshi (1991).

The ECD is a measure of efficiency with which absorbed food material is used in promoting the growth. This parameters significantly increased from third nymphal instar to adult in both the sexes and females had higher values than males. The

efficiency of conversion of ingested food (ECI) also increased with development and females were having higher value than males.

Hussain and Pfadt (1976) studied food utilization across developmental stages of big headed grasshopper (*Alucara elliotti* Thomas) and reported that the ingestion of food ranged from 27.8 to 215.3 mg insect^{-1} day^{-1} in males and from 26.2 to 411.8 mg insect^{-1} day^{-1} in females, suggesting a markedly greater intake of food compared to present investigation. This difference is reasonable shows that the values of ECD ranged between 6 to 26% in males and 4 to 39% in females of *A. elliotti* Thomas, indicating much poor conversion efficiency.

The utilization rates and assimilation efficiencies measured in the present study fall in the range reported by earlier workers for other grasshoppers species, (Smalley, 1960; Wiegert, 1965; Gyllenberg, 1969; Van Hook, 1971; Duke and Crossley, 1975; Singhal *et al.*, 1976; White, 1978; Delvi and Pandian, 1979; Kohler and Schaller, 1981; Vats and Mittal, 1983; Wiegert and Peterson, 1983; Kaushal and Vats, 1984; Kohler *et al.*, 1987; Kaushal and Joshi, 1991).

These finding suggest that *Xenocatantops karnyi* K. females are significantly larger than males and consume less biomass per unit of body weight. Female grasshoppers have a significantly higher tissue growth than males. Assimilation and tissue growth were positively related to the amount of food consumed. With increase in age and biomass, apparent digestibility declined but ECD & ECI increased.

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Seasonal Abundance of White Hairy Caterpillar *Thiacidas postica* Walker (Lymantriidae: Lepidoptera) On Ber

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ABSTRACT: The population of white hairy caterpillar *Thiacidas postica* Walker was noticed from July to October third week with a peak population in August first week. The population showed positive correlation with maximum and minimum temperatures and negative correlation with relative humidity and rainfall.

KEYWORDS: Ber, *Thiacidas postica* Walker, seasonal abundance.

INTRODUCTION

The ber *Ziziphus jujuba* is one of the most ancient and common fruits of India. Being a hardy fruit, it can be grown even on inferior and marginal lands which are unsuitable for growing other fruit crops. In view of good returns from ber, its cultivation is becoming increasingly popular in Andhra Pradesh. In Andhra Pradesh it is cultivated in the parts of Kurnool, Prakasam, Cuddapah, Chittoor, Ananthapur, Karimnagar and Rangareddy districts. But the major constraint for fruit production is the incidence of different pests.

The first three instar caterpillars of *Thiacidas postica* Walker are gregarious in habit. The larvae scrap the chlorophyll content of leaves, resulting in skeletonisation of leaves. The rest of the three instars feed oraceously on the entire leaves, leaving behind only the midribs and petioles resulting in severe defoliation. After defoliation caterpillars are found to occur on twigs also. Because this pest is severe on ber, studies were conducted on seasonal abundance to know the peak period of incidence and favourable weather factors for the incidence of *T. postica*.

Observations were made at weekly intervals on Gola variety starting from July 1999 to February 2000 from ten unprotected trees. In each tree, five tender twigs were selected at random covering all sides of the tree and the population of white hairy

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TABLE 1. Seasonal abundance of white hairy caterpillar *T. postica* Walker, on ber from July 1999 to February 2000

Standard week	Max. Temp. (°C)	Min. Temp. (°C)	R.H. %	R.F (mm)	Population of white hairy caterpillar (Larvae) (per 50 twigs)
			1999 July		
29	33.6	26.2	56.5	5.6	150
30	35.1	27.4	48.5	0.0	145
31	36.0	26.9	48.0	0.0	140
32	35.7	25.8	52.0	3.6	145
			August		
33	35.6	26.0	52.2	0.0	165
34	34.1	25.9	58.0	0.6	125
35	33.4	25.4	59.5	4.3	50
36	32.8	25.8	59.0	21.2	36
			September		
37	35.4	26.2	51.0	0.0	30
38	36.9	25.7	51.0	2.0	15
39	34.4	24.6	65.5	25.8	15
40	30.8	22.3	76.0	13.9	12
			October		
41	32.4	21.9	70.0	5.8	4
42	30.1	23.1	80.0	5.6	4
43	31.1	23.2	77.5	31.9	6
44	31.8	22.3	64.5	0.0	0
			November		
45	31.1	18.8	62.0	0.4	0
46	30.1	17.5	54.5	0.0	0
47	26.7	21.0	87.5	82.4	0
48	28.4	20.5	76.0	68.9	0
			December		
49	28.7	17.2	68.5	0.0	0
50	28.5	19.1	64.0	0.0	0
51	27.0	18.1	71.5	30.2	0
52	27.7	18.6	74.5	1.5	0
			January		
1	28.2	17.2	70.5	0.0	0
2	29.4	17.8	71.0	0.0	0
3	31.6	16.4	66.0	0.0	0
4	30.3	15.9	71.0	0.0	0
			February		
5	30.3	15.8	62.0	0.0	0
6	30.9	23.4	64.0	0.0	0
7	32.7	20.3	61.0	0.0	0
8	30.6	22.2	71.5	29.4	0

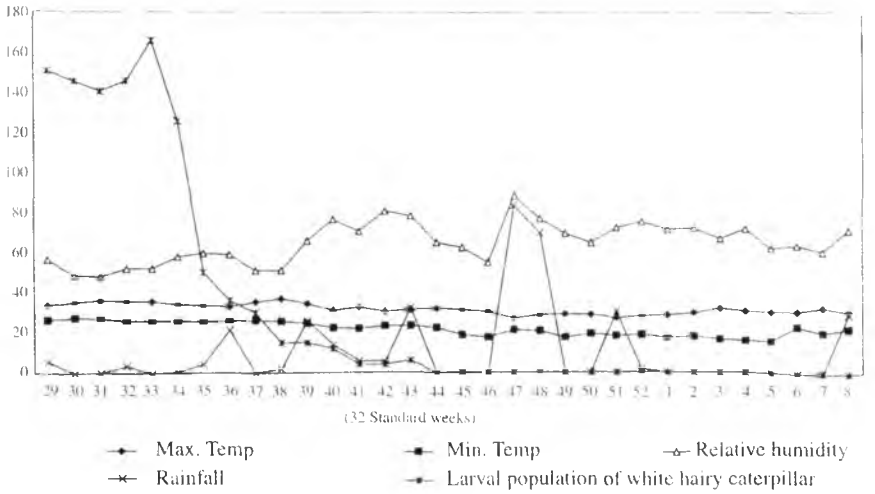

PLATE 1. Seasonal aubdance of white hairy caterpillar on ber.

TABLE 2. Relationship of population of white hairy caterpillar with weather factors

Sl. No.	Particulars	Correlation co-efficent	Partial regression coefficient	't' values
1.	Population of white hairy caterpillar and average maximum temperature	0.66163	0.147	−0.739 NS
2.	Population of white hairy caterpillar and average minimum temperature	0.70063	0.012	2.684 *
3.	Population of white hairy caterpillar and average relative humidity	−0.65362	0.1072	−2.216 *
4.	Population of white hairy caterpillar and average rain fall	−0.19893	0.1275	−0.132 NS

caterpillar was noted. Weather parameters *viz.*, maximum and minimum temperatures, relative humidity, rainfall were recorded continuously during the period of study. Population data was correlated with the weather parameters to know the influence of climatic variations of pest build up.

There was no population of white hairy caterpillar in the third week of October (43 standard week) and during November to February (45 to 52 and 1 to 8 standard weeks). The population first appeared from first week of July (29 standard week) and attained peak during August first week (Table 1) (Fig. 1) and decreased slowly from second week of August (34 standard week). A minimum population of 14 caterpillars per 50 twigs was recorded in October month (41 to 44 standard weeks). Mehra and Sah (1970) reported that the pest was active from March to November.

Correlation and regression between population counts and climatic factors *viz.*,

maximum temperature, minimum temperature, relative humidity and rainfall were worked out and coefficient are given in Table 2. The correlation of population count with maximum temperature ($r = 0.66163$) was positive and non-significant. Population count with minimum temperature ($r = 0.70063$) was positive and significant. Correlation between relative humidity ($r = 0.65362$) and population count of white hairy caterpillar was negative and significant, where as with rainfall ($r = 0.19893$) it was negative and non-significant.

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New Record of Yellow Hard Scale *Aspidiotus destructor* Sign. (Homoptera: Diaspididae) on Betelvine

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ABSTRACT: The circular hard scale *Aspidiotus destructor* Sign. was recorded on betelvine and it was reported for the first time in India.

KEYWORDS: Hard scale, *Aspidiotus destructor*.

Piper betle L. (Piperaceae) a climbing shrub is cultivated for its leaves, which are used as masticatory and is a popular commercial crop in cauvery delta of Tamil Nadu. The major insects causing damage are the scale insect *Lepidosaphes cornutus* Ramk and red spider mite *Tetranychus cinnabarinus* Boisdual (Muthukrishnan and Gopalan, 1965).

At Sugarcane Research Station, Sirugamani, betelvine leaves from tender shoots showed wilting symptom during June 1998. A large number of yellow hard scale, *Aspidiotus destructor* Sign. were found congregated particularly on the upper leaf area. The insect suck the sap from leaf tissues resulting in yellowing and wilting. There was neither the ants nor the sooty mould on these plants.

The adult female measures about 0.9126 ± 0.0194 mm in length and 0.8397 ± 0.0136 mm in width. It is oval in shape and yellow coloured, antenna vestigeal in the form of a tubercle with a small hair or spine like projection (Fig. 1). Anterior and posterior spiracles present; perivulvar pores present in four groups of four to seven pores each. Pygidium compressed with three pairs of prominent lobes (Fig. 2).

This hard scale is known to attack coconut, castor, brinjal, banana, citrus, guava, mango, tamarind, pepper, arecapalm, rubber and tea (Ayyar, 1963). It is considered as a major pest on coconut. However, this is the first record of *A. destructor* on betelvine.

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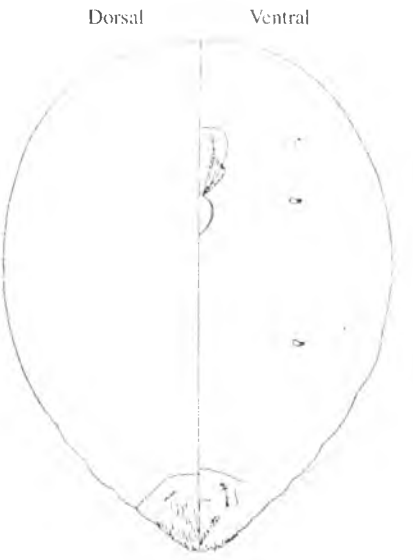

PLATE 1. General features of adult female.

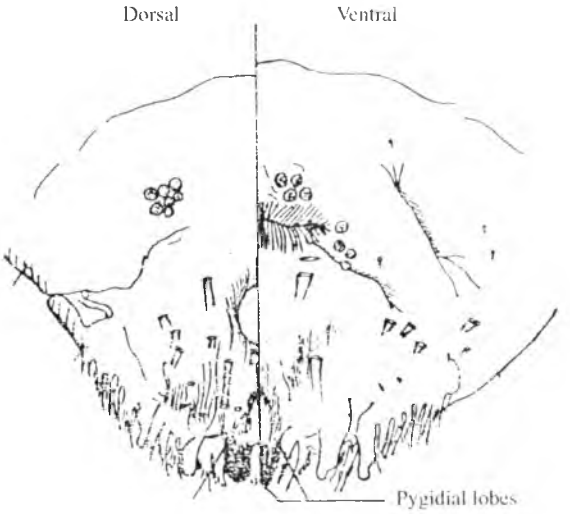

PLATE 2. Pygidium of adult female.

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